

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A01H 5/00, C07H 21/04, C12N 5/14, 15/29, 15/52, 15/82		A1	(11) International Publication Number: WO 00/67558 (43) International Publication Date: 16 November 2000 (16.11.00)
(21) International Application Number: PCT/US00/12450 (22) International Filing Date: 5 May 2000 (05.05.00) (30) Priority Data: 60/132,919 6 May 1999 (06.05.99) US (71)(72) Applicant and Inventor: TIMKO, Michael [US/US]; 1610 Old Ballard Road, Charlottesville, VA 22901 (US). (74) Agent: HANSEN, Christine, M.; Connolly Bove Lodge & Hutz LLP, 1210 Market Street, P.O. Box 2207, Wilmington, DE 19899 (US).			(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: REGULATION OF GENE EXPRESSION IN TOBACCO FOR MANIPULATION OF PLANT GROWTH AND SECONDARY METABOLISM			
(57) Abstract This invention relates to enzymes involved in alkaloid, and specifically nicotine, formation in tobacco plants. The invention is based, at least in part, on the nucleotide sequences encoding four variants of putrescine N-methyltransferase (PMT1, PMT2, PMT3, and PMT4), two variants of arginine decarboxylase (ADC1 and ADC2), ornithine decarboxylase (ODC), S-adenosylmethionine synthetase (SAMS), a fragment of NADH dehydrogenase, and a fragment of phosphoribosylanthranilate isomerase. The invention also relates to proteins expressed by these nucleotides, promoter regions of these nucleotides, use of these promoter regions to culture transgenic plant cells and to produce transgenic plants, sense and antisense nucleotides complementary to all or portions of these nucleotide sequences, use of sense and antisense nucleotides to regulate gene expression, and assays using proteins involved in alkaloid formation in tobacco plants.			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

REGULATION OF GENE EXPRESSION IN TOBACCO FOR MANIPULATION OF PLANT GROWTH AND SECONDARY METABOLISM

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application is a continuation-in-part of US Patent Application Ser. No. 60/ 132,919, filed May 6, 1999, now abandoned, which is hereby incorporated by reference in its entirety herein.

FIELD OF THE INVENTION

10 This invention relates to enzymes involved in alkaloid, and specifically nicotine, formation in tobacco plants. The invention is based, at least in part, on the nucleotide sequences encoding four variants of putrescine N-methyltransferase (PMT1, PMT2, PMT3, and PMT4), two variants of arginine decarboxylase (ADC 1 and ADC2), ornithine decarboxylase (ODC), S-adenosylmethionine synthetase (SAMS), a fragment of NADH dehydrogenase, and a fragment of
15 phosphoribosylanthranilate isomerase. The invention also relates to proteins expressed by these nucleotides, promoter regions of these nucleotides, use of these promoter regions to culture transgenic plant cells and to produce transgenic plants, sense and antisense nucleotides complementary to all or portions of these nucleotide sequences, use of sense and antisense nucleotides to regulate gene expression, and assays using proteins involved in alkaloid formation in tobacco plants.

BACKGROUND OF THE INVENTION

I. Alkaloid Formation

20 Alkaloids are one of the most diverse groups of secondary compounds found in plants and they are the product of a complex biosynthesis pathway (Hashimoto and Yamada, 1994; Chou and Kutchan, 1998; Waterman, 1998). Why plants accumulate these compounds and in so many
25 different forms is not known. Moreover, for many alkaloids, the exact site of synthesis and the factors that control their intercellular distribution and accumulation remain to be determined (Hashimoto and Yamada, 1994; Kutchan, 1995; Chou and Kutchan, 1998).

30 Nicotine is the most abundant alkaloid present in cultivated tobacco. Nicotine is formed primarily in the roots of the tobacco plant and subsequently is transported to the leaves, where it is stored (Tso, Physiology and Biochemistry of Tobacco Plants, pp. 233-34, Dowden, Hutchinson & Ross, Stroudsburg, Pa. (1972)).

The synthesis and accumulation of nicotine and other tobacco alkaloids are known to be controlled by various developmental, environmental, and chemical cues. Changes in phytohormone

(e.g., auxin, cytokinin) levels and/or ratios as a consequence of developmental age (Hashimoto and Yamada, 1994; Kutchan, 1995) or by direct manipulation of plant cell culture conditions have been shown to affect the synthesis and accumulation of nicotine and various tobacco alkaloids (Hashimoto and Yamada, 1994; Hibi *et al.*, 1994; Eilbert, 1998). Various abiotic factors (wounding, drought stress, pH imbalance, etc.) [Hashimoto and Yamada, 1994; Kutchan, 1998; Waterman, 1998] 1, 2, 4], as well as biotic factors, such as herbivory, insect feeding, and attack by various microbial and fungal pathogens, are known elicit increased production of nicotine and other alkaloids in the leaves of wild and cultivated tobacco species (Baldwin, 1989; Saito and Murakoishi, 1998; Baldwin and Prestin, 1999). In addition, the commercial practice of topping (i.e., removal of flowering head and young leaves at the upper portions of the plant), results in increases in nicotine and the amount and complexity total alkaloids present in the leaves of *Nicotiana tabacum* (Hashimoto and Yamada, 1994; Hibi *et al.*, 1994). The factors controlling the topping-induced increase in alkaloid biosynthesis are not known, but likely involve a complex physiological response in the plant as a result of altered phytohormones and wound induced signaling (Akehurst, 1981; Hibi *et al.*, 1994; Kutchan, 1998). In this regard, considerable evidence now exists indicating that a jasmonic acid (JA)- mediated signal transduction pathway may play a role in regulation of gene expression contributing to this increase in alkaloid biosynthesis (Baldwin *et al.*, 1994, 1996, 1997; Ohnmeiss *et al.*, 1997; Imanishi *et al.*, 1998a, 1998b).

The nicotine molecule is comprised of two heterocyclic rings, a pyridine moiety and a pyrrolidine moiety, each of which is derived from a separate biochemical pathway. The pyridine moiety of nicotine is derived from nicotinic acid. The pyrrolidine moiety of nicotine is provided through a pathway leading from putrescine to N-methylputrescine and then to N-methylpyrroline. (Goodwin and Mercer, Introduction to Plant Biochemistry, pp. 488-91, Pergamon Press, New York, (1983)).

Putrescine is formed in plants by one of two pathways (Chattopadhyay and Ghosh, 1998). It can be synthesized directly from ornithine, in a reaction catalyzed by the enzyme ornithine decarboxylase (ODC, EC 4.1.1.17), or formed indirectly from arginine in a reaction sequence initiated by arginine decarboxylase (ADC, EC 4.1.1.19). Putrescine formed by the ADC and/or ODC pathway serves as precursor in the synthesis of the higher polyamines, spermine and spermidine, catalyzed by the enzymes spermine synthase and spermidine synthase, respectively, or it is converted to N-methylputrescine by the action of putrescine N-methyltransferase (PMT), the first committed step in nicotine biosynthesis (Hashimoto and Yamada, 1994; Kutchan, 1995; Chattopadhyay and Ghosh, 1998). N-methyl putrescine is oxidized by a diamine oxidase and cyclized to form the 1-methyl- Δ^1 -pyrrolium cation, which is condensed with nicotinic acid or its derivative to form nicotine

(Hashimoto and Yamada, 1994).

Putrescene is a precursor for N-methylputrescine, which then forms N-methylpyrroline. Conversion of putrescine to N-methylputrescine is catalyzed by the enzyme putrescine N-methyltransferase ("PMT"), with S-adenosylmethionine serving as the methyl group donor. PMT appears to be the rate-limiting enzyme in the pathway supplying N-methylpyrroline for nicotine synthesis in tobacco (Feth et al., "Regulation in Tobacco Callus of Enzyme Activities of the Nicotine Pathway", *Planta*, 168, pp. 402-07 (1986); Wagner et al., "The Regulation of Enzyme Activities of the Nicotine Pathway in Tobacco", *Physiol. Plant.*, 68, pp. 667-72 (1986)).

10 II. TRANSGENIC PLANTS

The methods of nicotine formation in tobacco and the genes involved have been studied both to better understand differential gene expression during tobacco growth and development, and also to discover tools useful for creating transgenic plants. For example, the regulatory sequences that modify protein expression in tobacco may be useful in creating transgenic tobacco or other transgenic plants.

It has already been demonstrated that tissues of many plant species may be transformed by exogenous, typically chimeric, genes which are effective to stably transform cells of the tissues. For several species, tissues transformed in this fashion may be regenerated to give rise to whole transgenic or genetically engineered plants. The engineered traits introduced into the transgenic plants by these techniques have proven to be stable and have also proven to be transmissible through normal Mendellian inheritance to the progeny of the regenerated plants. One such desirable trait is the production in the plant cells of desired gene products in vivo in the cells of the transgenic plants. For a chimeric gene to be effective, the foreign DNA sequence containing a coding region should be flanked by appropriate promotion and control regions. Commonly used plant cell transcription promoters include the nopaline synthase promoter from the T-DNA of *A. tumefaciens* and the 35S promoter from the cauliflower mosaic virus.

In order for the newly inserted chimeric gene to express the protein for which it codes in the plant cell, the proper regulatory signals must be present and in the proper location with respect to the gene. These regulatory signals include a promoter region, a 5' non-translated leader sequence and a 3' polyadenylation sequence. A promoter is a DNA sequence that directs the cellular machinery of a plant to produce RNA from the contiguous structural coding sequence downstream (3') to the promoter. The promoter region influences the rate at which the RNA product of the gene and resultant protein product of the gene is made. The 3' polyadenylation signal is a non-translated region that functions in

the plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA to enable the mRNA to be transported to the cytoplasm and to stabilize the mRNA for subsequent translation of the RNA to produce protein.

Other plant cell transformation techniques are directed toward the direct insertion of DNA into the cytoplasm of plant cells from which it is taken up, by an uncharacterized mechanism, into the genome of the plant. One such technique is electroporation, in which electric shock causes disruption of the cellular membranes of individual plant cells. Plant protoplasts in aqueous solution when subject to electroporation will uptake DNA from the surrounding medium. Another technique involves the physical acceleration of DNA, coated onto small inert particles, either into regenerable plant tissues or into plant germline cells.

The availability of cloned nucleic acid sequences encoding an enzyme involved in alkaloid synthesis allows for the potential manipulation of alkaloid contents. Furthermore, the availability of promoters useful for expressing genes in plants allows for the creation of chimeric molecules and transgenic plants, which in turn result in possible native plant production of desirable proteins.

Previously reported work discloses cloning nucleotides encoding proteins involved in the biosynthesis of nicotine, and isolating such proteins. Approximately twenty or more cDNAs and/or genomic DNA fragments encoding different enzymes involved with alkaloid formation have been isolated (Chattopadhyay and Ghosh, 1998). For example, successful cloning of partial or full-length cDNA encoding ODC activity from tobacco was disclosed by (Malik *et al.*, *J. Plant Biochem. & Biotech.* 5:109-112 (1996)). Also, a relatively crude preparation of PMT (30-fold purification) has been subjected to limited characterization (Mizusaki *et al.*, "Phytochemical Studies on Tobacco Alkaloids XIV. The Occurrence and Properties of Putrescine N-Methyltransferase in Tobacco Plants", *Plant Cell Physiol.*, 12, pp. 633-40 (1971)). A process for purifying PMT is disclosed in US Patent No. 5,369,023, "Method of purifying putrescine n-methyltransferase from tobacco plant extract with an anion exchange medium", hereby incorporated by reference in its entirety herein. Several laboratories have reported the cloning of partial or full-length cDNAs encoding ADC (Bell and Malmberg, 1990; Rostogi *et al.*, 1993; Perez-Amador *et al.*, 1995; Nam *et al.*, 1997; Watson and Malmberg, 1996). Comparisons of the amino acid sequences of ADC from various plants revealed a high degree of conservation among the various proteins, as well as homology to ODC (Malmberg *et al.*, 1998).

It is an object of the present invention to characterize the nucleotide and amino acid sequences of enzymes involved in the biosynthesis of nicotine in tobacco. It is also an object of the present invention to provide plant promoter regions that are capable of conferring high levels of transcription in rapidly dividing cells of transformed plants when coupled with a heterologous coding

sequence in a chimeric gene. Further, the invention is directed to chimeric genes incorporating such promoter regions, stable transfection of plants with these chimeric genes, and the plants and cells that are transfected, as well as seeds of such transfected plants. It is a further object to characterize sense and antisense nucleotides capable of regulating expression of genes encoding for enzymes involved in the biosynthesis of alkaloids.

SUMMARY OF THE INVENTION

Proteins involved in the biosynthesis of nicotine in tobacco *N. tabacum* are the subject of this invention. More specifically, the invention concerns four variants of putrescine N-methyltransferase (PMT1, PMT2, PMT3, and PMT4), two variants of arginine decarboxylase (ADC 1 and ADC2), ornithine decarboxylase (ODC), S-adenosylmethionine synthetase (SAMS), NADH dehydrogenase, and phosphoribosylanthranilate isomerase.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Genomic DNA gel blot analysis of the PMT gene family in *N. tabacum* cv. Xanthi. Total genomic DNA (30 µg) was digested with *Kpn*I, *Eco*RI, or *Eco*RI and *Kpn*I, separated by agarose gel electrophoresis, and transferred to nylon membranes. The membrane was hybridized with a ³²P-labeled antisense strand probe covering the complete coding region of the *NtPMT1a* cDNA. Identity of the hybridizing bands as determined by comparison to phage DNA digests is indicated. Molecular weights are given in kb. Note that *Kpn*I shifts only the *NtPMT1b* band in the gel blot because this restriction site is present only in Exon 1 of *NtPMT1b* and not *NtPMT1a*.

Figure 2. Amino acid sequence alignment of *N. tabacum* PMTs. Shown is a PILEUP alignment of the predicted amino acid sequences of the various tobacco PMTs. Amino acid residues that differing among the PMTs are shaded. *NtPMT1a*, *NtPMT2*, *NtPMT3*, and *NtPMT4* refer to the deduced amino acid sequences of the PMTs encoded by the *NtPMT1a*, *NtPMT2*, *NtPMT3*, and *NtPMT4* genes, respectively, isolated from *N. tabacum* cv. Xanthi genomic DNA; *cNtPMT1a* is the predicted amino acid sequence of the A411 cDNA (Accession No. D28506) isolated from *N. tabacum* cv. Burley 21 by Hibi *et al.* (1994). The location of the exon-intron boundaries are indicated by the dark vertical line. The nucleotide sequences for *NtPMT1a*, *NtPMT2*, *NtPMT3*, and *NtPMT4* appear in GenBank under the accession numbers AF126810, AF126809, AF126811, and AF126812, respectively

Figure 3. Polyacrylamide gel electrophoresis analysis of PCR amplified genomic DNA fragments

encoding Exon 1 of PMT from various species of *Nicotiana*. PCR amplification was carried out as described in the Materials and Methods using Exon 1-specific primers 1 and 2 and total genomic DNA isolated from *N. tabacum*, *N. glauca*, and *N. glauca*. The amplification products were separated by electrophoresis on 6.5% polyacrylamide gels, the gels fixed, and subject to
 5 autoradiography. The amplification products isolated from *N. tabacum* cv. Burley 21 and *N. tabacum* cv. Xanthi were identical and only the amplification products from the reactions with *N. tabacum* cv. Burley 21 DNA are shown. Standards were generated in identical reaction conditions primed with plasmid DNA encoding the various *PMT* genes isolated in this study.

10 *Figure 4.* Nucleotide sequence alignment of the 5'-flanking regions of the *N. tabacum* *PMT* genes. Shown is a PILEUP alignment of the nucleotide sequences upstream of the initiating methionine (MET) codon of the four *PMT* genes isolated from *N. tabacum* cv. Xanthi. The proposed start site for transcription of the *NtPMT1a* gene is indicated by the +1 above the sequences. The TATA-box and CCAAT-box motifs are boxed. Potential transcriptional regulatory elements identified by
 15 MOTIF search programs are also boxed and indicated by the following abbreviations: PAL: palindromic sequences; G-Box: G-Box homologous sequences; MRE: metal-responsive element homolog. Nucleotides identical in three or more sequences are shaded. The polyguanine-rich region is underlined. Numbering is indicated to the right and is relative to the proposed start site
 20 of each gene.

Figure 5. RNA gel blot analysis of *PMT* transcript levels in various tissues. Total RNA was isolated from various tissues of mature *N. tabacum* cv. Burley 21 and analyzed by gel blot analysis using a ³²P-labeled *NtPMT1a* cDNA coding region (Exons 2 to 8) probe capable of detecting all *PMT* transcripts.

- 25 A. *PMT* transcript levels in various tobacco plant tissues and/or organs.
 B. Induction of *PMT* expression in tobacco roots following topping. Abbreviations: HP, wild-type (*Nic1Nic2*) Burley 21; LP, low alkaloid (*nic1nic2*) mutant. The β -subunit of mitochondrial ATPase (β -ATPase) served as a control.

30 *Figure 6.* Semi-quantitative RT-PCR analysis of *PMT* gene expression in roots of tobacco plant before and after topping.

- A. Shown is relative abundance of the individual *PMT* gene transcripts before and after topping. RT-PCR was carried out as described in the Material and methods using Exon 1 specific primers. Messenger RNA was amplified from total RNA isolated from the roots of wild-type (HP,

Nic1Nic2) Burley 21 and low alkaloid (LP, *nic1nic2*) Burley 21 tobacco plants. Far right lane represents size standards for the genes isolated by PCR amplification from plasmid DNA. The β -subunit of mitochondrial ATPase (β -ATPase) served as a control.

- 5 B. Bar graphs showing relative expression of the individual PMT genes following topping in both HP and LP tobacco roots. Abbreviations: HP, wild-type (*Nic1Nic2*) Burley 21; LP, low alkaloid (*nic1nic2*) mutant.

10 **Figure 7.** The nucleotide and predicted amino acid sequences of the transcribed portions of the *N. tabacum* cv Xanthi NtADC1 and NtADC2 genes. Shown are the complete nucleotide and predicted amino acid sequence of the *N. tabacum* cv Xanthi NtADC1 gene and where it differs from the NtADC2 gene sequence. The dots indicate nucleotide sequence identity and the stars indicate amino acid sequence identity. The proposed polyadenylation signal is underlined. The sequences terminate at the point of polyadenylation found in the full length cDNA (Wang, 1999; AF127239). The
15 complete nucleotide sequences for the *N. tabacum* cv Xanthi NtADC1 (AF127240) and NtADC2 (AF127241) including the 5' and 3' flanking sequences appear in Genbank.

Fig. 8. Comparison of the predicted amino acid sequences of arginine decarboxylases (ADCs) from various species. Shown is a PILEUP alignment of the predicted amino acid sequence of the *N. tabacum* cv Xanthi NtADC1 gene (AF127240) aligned to the predicted ADC protein sequences from
20 *N. sylvestris* (AB12873), *Arabidopsis thaliana* (AF009647), *Avena sativa* (oat) (X56802), *Lycopersicon esculentum* (tomato) (L16582) and *Escherichia coli* (M31770). Amino acid residues conserved among the various ADC are shaded.

25 **Fig. 9.** Gel blot analysis of ADC transcript levels in the roots of wild-type and low alkaloid mutant Burley 21 tobacco before and after topping. Total RNA was isolated from the roots of mature wild-type and low alkaloid mutant *N. tabacum* cv. Burley 21 and analyzed by gel blot analysis using [α - 32 P]-dCTP labeled probes recognizing the coding region of ADC or the β -subunit of tobacco mitochondrial ATP synthase (Boutry and Chua, 1985). Quantitation was carried out by
30 phosphorimaging using a Molecular Dynamics PhosphorImager. Values were normalized relative to the intensities of the *atp2* control band in each lane. The experiment was conducted twice with different total RNA samples.

Fig. 10. Differential expression of NtADC-1 and NtADC-2 in various tissues of tobacco. Expression of the NtADC-1 and NtADC-2 genes was analyzed using semi-quantitative RT-PCR and gene specific primers capable of discriminating between transcripts arising from the two genes. Panel A shows control reactions demonstrating primer specificity in the PCR reactions using plasmids containing the NtADC-1 and NtADC-2 coding sequences. The numbers above the lane refer to the specific primer combinations as described in the Material and methods. Panel B shows the results of RT-PCR reactions using first strand cDNA synthesized from total RNA extracted from either root, leaf, or flowers. As an internal control, primers specific for the *atp2* gene transcript were included in the amplification reactions. All reactions were carried out within the linear range of template amplification as determined by varying template amount, amplification time, and temperature as described in Riechers and Timko (1999).

Fig. 11. Genomic DNA gel blot analysis of the ODC gene family in *N. tabacum*. Total genomic DNA (30 μ g) was digested with *Eco*RI or *Hind*III, fractionated by agarose gel electrophoresis, transferred to nylon membranes and hybridized with an α -³²P-dCTP labeled probe encoding full-length ODC cDNA as described in the Materials. The mobility of molecular weight standards are given to the right of the figure in kilobases (kb).

Fig. 12. Comparison of the nucleotide and predicted amino acid sequences of the *NtODC-1* and *NtODC-2* genes. Shown are the nucleotide and predicted amino acid sequences of the *NtODC-1* (AF233850) and *NtODC-2* (AF233849) genes. In the figure, the complete amino acid sequence of the pODC2 is given and the pODC1 sequence is given only where it differs. The start site of transcription is designated as +1 and the poly(A) addition site is indicated by the arrow. Within the relevant regions of homology, nucleotide differences between the *NtODC-1* and *NtODC-2* genes are in bold lettering. The proposed TATA-box, and polyadenylation signal are shaded.

Fig. 13. Protein sequences alignment of ornithine decarboxylases (ODCs) from various species. Shown is a PILEUP alignment of the predicted amino acid sequences of the *N. tabacum* cv. Xanthi pODC2 protein (AF233849) with the ODCs from *N. tabacum* cv. SC58 (Y10472) and cv. BY-2 (ABO31066), *Lycopersicon esculentum* (tomato) (AF030292), *Datura stramonium* (jimsonweed) (X87847), *Saccharomyces cerevisiae* (NP_012737), and humans (*Homo sapiens*; AAA59966). Amino acid residues conserved among the various ODCs are shaded.

Fig. 14. Gel blot analysis of *ODC* transcript levels in various tissues of mature tobacco plants and in the roots before and after topping. Total RNA was isolated from various tissues of mature *N. tabacum* cv. Burley 21 and analyzed by gel blot analysis using an α -³²P-dCTP labeled coding region probes for ODC. (A) Transcript levels in various organs of wild-type tobacco: R, root; S, stem; L, leaf; SE, sepal; PE, petal; O, ovary; S, stamen; and AN, anther. (B) Transcript levels in roots of Burley 21 tobacco plants before and after topping. RNA gel blot analysis of the tissues-specific distribution and post-topping expression of transcripts encoding ODC in tobacco. As a control, the blots were also probed with radioactively labeled probes encoding the alkaloid biosynthesis enzyme putrescine N-methyltransferase (PMT) and a root specific β -glucosidase (TBG-1).

DETAILED DESCRIPTION OF THE INVENTION

Nucleic acid sequences have been isolated from tobacco that encode important enzymes in nicotine and total alkaloid formation, including PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, and SAMS. Also identified are cDNA fragments encoding partial segments of NADH dehydrogenase and phosphoribosilanthronilate isomerase. Also identified are promoter regions for the nucleotides encoding PMT1, PMT2, PMT3, PMT4, and ADC2. All of these nucleic acids are isolated from *Nicotiana tabacum* L.

"Promoter" and "promoter region" are terms used interchangeably herein to refer to a DNA sequence that regulates expression of a selected DNA sequence operably linked to the promoter, and which effects expression of the selected DNA sequence in cells. The term also encompasses the 5'untranslated region that may be transcribed into mRNA but is not translated.

"Protein", "polypeptide", and "peptide" are used interchangeably herein when referring to a gene product.

In one aspect, the invention features isolated nucleic acid molecules encoding for PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, and SAMS, a fragment of NADH dehydrogenase and a fragment of phosphoribosilanthronilate isomerase. The disclosed molecules can be non-coding (e.g. probe, antisense or ribozyme molecules) or can code for a functional enzyme. In one embodiment, the nucleic acid molecules can hybridize to the nucleic acid sequences encoding for PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, SAMS, a fragment of NADH dehydrogenase, or a fragment of phosphoribosilanthronilate isomerase or to the complements of these nucleic acid sequences. In a preferred embodiment, the hybridization is conducted under mildly stringent or stringent conditions.

In further embodiments, the nucleic acid molecule is at least 50%, 60%, 70%, 80% and more preferably at least 90% or 95% homologous in sequence to the nucleic acid sequences encoding for PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, SAMS, a fragment of NADH dehydrogenase, or

a fragment of phosphoribosilanthronilate isomerase or to the complements of these nucleic acid sequences. In another embodiment, the nucleic acid encodes a polypeptide that is at least 50%, 60%, 70%, 80% and more preferably at least 90% or 95% similar in sequence to the amino acid sequence of PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, SAMS, the fragment disclosed herein of
5 NADH dehydrogenase, or the fragment of phosphoribosilanthronilate isomerase disclosed herein.

In another embodiment, the invention features isolated polypeptides, preferably substantially pure preparations, encoded for by the nucleic acid sequences of the invention. Particularly preferred are those polypeptides encoded for by the nucleic acid sequences identified by (SEQ. ID. NO. 2), (SEQ. ID. NO. 5), (SEQ. ID. NO. 8), (SEQ. ID. NO. 11), (SEQ. ID. NO. 13), (SEQ. ID. NO. 15),
10 (SEQ. ID. NO. 18), (SEQ. ID. NO. 21), (SEQ. ID. NO. 23), (SEQ. ID. NO. 25) or (SEQ. ID. NO. 26) or comprising a nucleotide sequence encoding the amino acid sequence encoded by (SEQ. ID. NO. 3), (SEQ. ID. NO. 6), (SEQ. ID. NO. 9), (SEQ. ID. NO. 12), (SEQ. ID. NO. 14), (SEQ. ID. NO. 16), (SEQ. ID. NO. 19), (SEQ. ID. NO. 22) or (SEQ. ID. NO. 24). In particularly preferred embodiments, the subject polypeptides can aid in regulating the production of alkaloids, particularly
15 nicotine, in plants. In one embodiment, the polypeptide is identical to or similar to the protein represented by the amino acid sequences of (SEQ. ID. NO. 3), (SEQ. ID. NO. 6), (SEQ. ID. NO. 9), (SEQ. ID. NO. 12), (SEQ. ID. NO. 14), (SEQ. ID. NO. 16), (SEQ. ID. NO. 19), (SEQ. ID. NO. 22) or (SEQ. ID. NO. 24). In a preferred embodiment, the polypeptide is encoded by a nucleic acid that hybridizes with a nucleic acid represented in.

20 The polypeptides of the present invention can comprise full length proteins, such as represented by (SEQ. ID. NO. 3), (SEQ. ID. NO. 6), (SEQ. ID. NO. 9), (SEQ. ID. NO. 12), (SEQ. ID. NO. 14), (SEQ. ID. NO. 16), (SEQ. ID. NO. 19), (SEQ. ID. NO. 22) and (SEQ. ID. NO. 24), or can comprise one or more fragments corresponding to one or more particular motifs/domains, or to arbitrary sizes, e.g., at least 5, 10, 25, 50, 100, 150, or 200 amino acids in length.

25 Another aspect of the invention features chimeric genes comprised of a promoter for the genes for PMT2, PMT1, PMT3, PMT4, or ADC2. Yet another aspect of the invention features chimeric genes or chimeric molecules comprised respectively of the functional gene encoding for or the protein PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, SAMS, NADH dehydrogenase and/or phosphoribosilanthronilate isomerase.

30 The invention also concerns isolated and purified promoter regions for tobacco Beta-glucosidase and their use in chimeric genes or chimeric molecules.

Another aspect of the invention involves vectors capable of transporting another nucleic acid to which a vector has been linked. Preferably, the vectors comprise the nucleic acid sequences of the invention or their complements.

The invention also features transgenic plants and their seeds that include (and preferably express) a heterologous form of PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, SAMS, NADH dehydrogenase and/or phosphoribosilanthronilate isomerase. The present invention also encompasses transgenic plants that contain in their genome a chimeric gene construction incorporating the nucleic acid encoding PMT1, PMT2, PMT3, PMT4, ADC1, ADC2, ODC, SAMS, NADH dehydrogenase and/or phosphoribosilanthronilate isomerase. Such transgenic plants and their seeds may be useful to natively produce enhanced quantities of desirable exogenous proteins, such as compounds useful for pharmaceutical purposes, or proteins that provide herbicide resistance.

Another feature of the invention is the use as probes of the DNA sequences disclosed herein or oligonucleotide fragments thereof. Probes may be useful to obtain additional gene family members or locate homologous genes in tobacco or other plant species. Copies of related genes can be obtained from existing genomic libraries or the genomic libraries can be constructed. In one embodiment, an isolated DNA sequence comprising about a fifteen to about a twenty-five base pair oligonucleotide sequence identical to any consecutive about fifteen to about twenty-five base pair sequence found in the sequences of the invention is used as a probe.

Another feature is use of the polypeptides of the invention in an assay, such as an assay to identify modulators of enzyme activity in plants.

Other features and advantages of the invention will be apparent to those of skill in the art.

The nucleotide and amino acid sequences of the invention are disclosed herein in the Sequence Listing, text, and the figures. Specific sequences of the invention are provided in the attached Sequence Listing and can be understood to represent promoters, nucleic acids, and proteins respectively relating to the following proteins: PMT2 (SEQ. ID. NOS. 1, 2, and 3); PMT1 (SEQ. ID. NOS. 4, 5, and 6); PMT3 (SEQ. ID. NOS. 7, 8, and 9); PMT4 (SEQ. ID. NOS. 10, 11, and 12); SAMS (SEQ. ID. NOS. 13 and 14); ODC (SEQ. ID. NOS. 15 and 16); ADC1 (SEQ. ID. NOS. 17, 18, and 19); ADC2 (SEQ. ID. NOS. 20, 21, and 22); ADC1 mRNA (SEQ. ID. NOS. 23 and 24); NADH dehydrogenase (SEQ. ID. NO. 25); and PAI (SEQ. ID. NO. 26). If only two sequence identifiers are provided for a protein, those sequences represent the nucleic acid and the protein respectively. If three identifiers are provided, the identifiers represent promoter, genomic or cDNA nucleic acid, and peptide sequences, respectively. If only one identifier is provided, it represents a DNA fragment coding for the protein or portions of it.

For other reference, the sequences may be found at the following records in GenBank at the following Accession Numbers, which records are hereby incorporated in their entirety herein: AF126810 (NtPMT1); AF126809 (NtPMT2); AF126811 (NtPMT3); AF126812 (NtPMT4); AF176908 (NtomPMT)(*Nicotiana tomentosiformis*); AF76909 (NotoPMT)(*Nicotiana otophora*);

AF127239 (ADC); AF127240 (ADC1); AF127241 (ADC2); AF127242 (ODC); AF233849 (ODC2); AF233850 (ODC1); and AF127243 (SAMS).

The following experimental discussion is presented to better illustrate the invention.

I. PMT

The present invention features the characterization of four members of the nuclear gene family encoding PMT in tobacco *N. tabacum*. The nucleic acid sequences encoding PMT and the amino acid sequences for the proteins are reported herein and can also be found in the DDBJ, EMBL, and GenBank Nucleotide Sequence Databases under the accession numbers for *NtPMT1a*, *NtPMT2*, *NtPMT3*, and *NtPMT4* at AF126810, AF126809, AF126811, and AF126812, respectively. The complete coding region and immediate 5'- and 3'- flanking regions are characterized.

As the discussion below shows, all four PMT genes present in the *N. tabacum* genome are expressed in the roots of wild-type plants and differentially regulated in tobacco lines expressing either high or low total alkaloid contents.

Materials and Methods

Plant materials

Seeds of *N. sylvestris*, *N. otophora*, and *N. tomentosiformis* were obtained from the USDA-ARS national tobacco germplasm collection (Oxford, NC). *N. tabacum* cv. Burley 21 and *N. tabacum* cv. Xanthi seeds were kindly provided by Glenn Collins, University of Kentucky. Tobacco plants used for DNA isolation were grown in a soil:vermiculite mixture in the greenhouse under natural lighting conditions. Plants used for RNA extraction were grown in Moltan Plus (Moltan Co., Middleton, TN).

Gel blot analysis of genomic DNA

Young leaves were collected from greenhouse grown tobacco (*N. tabacum* cv. Xanthi) plants and total genomic DNA was isolated from freshly-harvested tissues using a modification of the CTAB extraction method (Dellaporta *et al.*, 1983). Approximately 30 µg of total DNA was digested with *EcoRI*, *KpnI*, or *EcoRI* and *KpnI* and the digestion products separated by electrophoresis through a 0.75% agarose gel. Restricted and size-fractionated DNA was denatured and transferred to Nytran+ nylon membranes (Schleicher and Schuell, Keene, NH) by capillary blotting in 0.4N NaOH overnight. Membranes were prehybridized in 0.25M Na₂HPO₄ (pH 7.4), 7% SDS, 1 mM Na₂EDTA

for at least 2 hr, then hybridized overnight at 65°C in the same buffer with 2-3 x 10⁶ cpm/mL of a ³²P-labeled single-stranded probe (antisense DNA strand). The probe was prepared by the method of Bednarczuk *et al.* (1991) using a primer (Table 1, primer 4) designed from the 3' end of the *NtPMT1a* coding region (Exon 8) and the full-length coding region of the *NtPMT1a* cDNA as template. The *NtPMT1a* cDNA was generated by RT-PCR using synthetic oligonucleotide primers based on the N- and C-terminal sequences of the A411 cDNA reported by Hibi *et al.* (1994) and RNA template isolated from *N. tabacum* cv. Burley 21 roots. Membranes were washed at a final stringency of 0.1 x SSC, 0.1% SDS at 65°C. Hybridizing bands were visualized by autoradiography and/or imaged using a Molecular Dynamics PhosphorImager (Model 445 SI, Sunnyvale, CA).

Genomic library construction and phage isolation

A library of *N. tabacum* cv. Xanthi genomic DNA fragments constructed in EMBL3 was purchased from Clontech (Palo Alto, CA) and a total of 1.1 x 10⁶ recombinant phage were screened by plaque hybridization using random-primed ³²P-labeled *NtPMT1a* cDNA as probe (Sambrook *et al.*, 1989). Prehybridization, hybridization, and washing conditions were as described above. Positive hybridizing phage were plaque purified by subsequent rounds of rescreening and DNA was prepared from 18 independently isolated phage. The phage DNA was characterized by restriction analysis and DNA gel blot analysis and fragments containing the sequences encoding PMT were subcloned into pBluescript KS vectors for further analysis.

Comparison of the hybridizing fragments present in the 18 recombinant phage to the hybridization pattern obtained by genomic DNA blot analysis indicated that only three of the *PMT* genes suspected to be present in the *N. tabacum* genome were recovered from the library screen. To obtain sequences encoding *NtPMT1a*, a subgenomic library was constructed from *N. tabacum* cv. Xanthi DNA. The library consisted of gel-purified 2.5-3.5 kb *EcoRI* fragments ligated into λ _ZAP II vector arms and packaged using Gigapack III Gold packaging extracts according to the manufacturer's instructions (Stratagene, La Jolla, CA). The primary library was amplified once in *E. coli* XL1-Blue MRF' strain (Stratagene) and screened as described above, except that a random-primed ³²P-labeled *NtPMT1a* cDNA Exon 1-specific probe was used (Table 1). Exon 1 had previously been amplified by PCR using primers 1 and 2 (Table 1) and the *NtPMT1a* cDNA as template. The recombinant phage that hybridized with the probe was isolated from the sublibrary by two more rounds of plaque purification, and the pBluescript phagemid containing the approximate 3.1 kb *EcoRI* genomic fragment with the *NtPMT1a* gene was excised from the λ _ZAP II phage vector using the *in vivo* excision protocol described by Stratagene.

DNA sequence analysis

Unless otherwise noted, DNA sequencing was performed with double-stranded plasmid DNA
5 templates using fluorescent dye terminator technology (dRhodamine Terminator Cycle Sequencing
Ready Reaction kit) on an ABI 310 DNA sequencer (Perkin-Elmer Applied Biosystems). For
analysis of PCR products, following electrophoretic separation of amplification reaction products,
the bands of interest were excised from the polyacrylamide gels, the DNA extracted using the
10 Quiagen Gel Extraction Kit, and the recovered DNA used as sequencing template. Sequencing was
performed using AmpliTaq DNA polymerase and fluorescent dye terminator technology (as
described above) and primers 1 and 2 (Table 1) specific for Exon 1. Nucleotide and amino acid
sequences were analyzed and aligned using either the Clustal method and Lasergene software
(DNASTar Inc., Madison, WI) or the PILEUP and ALSCRIPT (Genetics Computer Group, Madison,
15 WI) sequence analysis package (Version 9.0). Transcription factor binding site homologies were
identified in promoter DNA sequences by searching the transcription factor database using the GCG
program.

RNA gel blot analysis

20 For RNA analysis, roots and other tissues were harvested from mature wild-type (HP; *Nic1Nic2*) and
low alkaloid mutant (LP; *nic1nic2*) Burley 21 tobacco plants. For topping experiments, the stem was
cut and the top one-third of the plant was removed just prior to flower opening. Roots were
harvested just prior to topping (0 hr control) and at various times after decapitation. The tissue was
immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction and isolation.
25 Total RNA was isolated from vegetative organs and floral structures of HP and LP Burley 21
tobacco using the TRI-reagent (Molecular Research Center Inc., Cincinnati, OH) and quantified
spectrophotometrically by measuring *A*₂₆₀. Total RNA (5 µg) was electrophoresed through 1.2%
agarose gels (containing 0.4 M formaldehyde) and transferred to Nytran⁺ nylon membranes.
Following prehybridization the membranes were hybridized with a single-stranded *NtPMT1a* cDNA
30 antisense probe (corresponding to the antisense strand of Exons 2 to 8 of the *NtPMT1a* cDNA coding
region) as described above. As a control to quantify and normalize RNA levels in each lane, the blot
was hybridized with a 400-bp probe derived from the β-ATPase cDNA using primers 6 and 7 (Table
1) as described below.

Semi-quantitative RT-PCR analysis of individual PMT transcript levels

Total RNA (1 µg) extracted from the roots of HP and LP Burley 21 tobacco plants was reverse-transcribed into first-strand cDNA at 42°C using Superscript II reverse transcriptase (Gibco BRL) according to the manufacturer's protocol. Two gene-specific primers were employed in the reactions: primer 5 capable of recognizing Exon 3 of the *PMT* genes and primer 8 specific for Exon 8 of the nuclear gene encoding the β -subunit of mitochondrial ATPase from *N. plumbaginifolia* (*NpATP2.1*) and *N. sylvestris* (*NsATP2.1*) (Boutry and Chua, 1985; Lalanne *et al.*, 1998). The β -ATPase transcript served as an internal reference (constitutively-expressed control) to determine loading accuracy and to normalize expression levels (Kinoshita *et al.*, 1992). Following first strand cDNA synthesis, two sets of nested primers (0.4 µM each primer) were used to amplify the *PMT* and β -ATPase transcripts: primers 1 and 2 (Table 1) recognized Exon 1 in all five *PMT* transcripts and gave products ranging in size from 220 bp to 420 bp and primers 6 and 7 amplified an approximately 400-bp region encompassing a portion of Exons 6 to 8 of the β -ATPase coding region. Amplification was carried out for 25 cycles using the following reaction conditions: denaturation at 95°C for 1 min, primer annealing at 60°C for 35 sec, and extension at 72°C for 1.5 min; a final extension was conducted at 72°C for 6 min. Amplification products were radioactively labeled by spiking the PCR reaction with 10 µCi ³²P-dCTP. Aliquots of the PCR reaction were analyzed on a 6.5% non-denaturing polyacrylamide/1X TBE gel and electrophoresed at 600 volts. The reaction conditions were optimized to provide amplification of both *PMT* and β -ATPase transcripts in the linear range of the reaction by varying the levels of first strand cDNA template, annealing temperature, and number of cycles of amplification as described in Kinoshita *et al.* (1992). Molecular weight standards were prepared by PCR amplification using the same primers and protocol described above and plasmid DNA templates containing the *PMT* encoding genomic fragments, as well as genomic DNA from the various *Nicotiana* species indicated in the text.

Following electrophoresis, the polyacrylamide gels were fixed in 5% MeOH, 7.5% acetic acid for 30 min, dried overnight, and used to expose X-ray film. *PMT* band intensities were quantified using phosphorimager analysis (Molecular Dynamics) and normalized relative to the intensities of the β -ATPase control band in each lane. The experiment was conducted twice with different total RNA samples, and representative results are presented from one of the two experiments.

Results*PMT gene structure and organization in N. tabacum*

Gel blot analysis of total genomic DNA isolated from *N. tabacum* cv. Xanthi, hybridized with a radioactively-labeled cDNA (*NtPMT1a*) encoding the complete coding region of putrescine N-methyltransferase (PMT) showed the presence of five major hybridizing bands in *KpnI* or *EcoRI* digested DNA, consistent with the presence of a small multigene family in the *N. tabacum* genome (Figure 1).

As part of our initial characterization of the gene family encoding PMT in *N. tabacum*, an EMBL3 genomic library, prepared from *N. tabacum* cv. Xanthi DNA, was screened using the *NtPMT1a* (A411 homologous) cDNA as probe. From a total of 18 recombinant phage isolated, three phage were recovered that contained genomic fragments encoding the *NtPMT2*, *NtPMT3* and *NtPMT4* genes. The three *PMT* genes were completely encoded within a unique sized *EcoRI* fragment within the phage DNA insert which allowed for the correlation of each with a hybridizing restriction fragment on the gel blot of *N. tabacum* genomic DNA (Figure 1). The complete coding region and immediate 5' and 3' non-coding sequences of the three genes were determined and found to encode full-length PMT proteins (Figure 2). Each *PMT* gene consisted of 8 exons and 7 introns, consistent with the gene structure reported previously for the *PMT* genes from *N. sylvestris* (Hashimoto *et al.*, 1998a). Comparison of the deduced amino acid sequences (Figure 2) revealed that the encoded PMT proteins were extremely similar over their entire length, with the only significant variability in primary sequence localized to the extreme N-terminal region of the protein. This region, completely encoded within Exon 1, contains a variable number of an 11 amino acid repeat with a consensus sequence of NGHQNQTSEHQ. The function of the repeated sequence is unknown, but is apparently inconsequential to enzyme function, since the number of repeats does not influence activity and PMTs characterized from other species do not contain the repeated element (Hashimoto *et al.*, 1998a; Suzuki *et al.*, 1999a).

Multiple rounds of screening of the EMBL3 genomic library failed to yield additional hybridizing phage containing sequences encoding the other two *PMT* genes thought to be present in the *N. tabacum* genome and, therefore, a directed cloning approach was pursued using a subgenomic library constructed from *EcoRI* fragments isolated from *N. tabacum* cv. Xanthi. From this hybridization screening, a phage containing the approximately 3.1 kb *EcoRI* fragment encoding *NtPMT1a* was recovered. The coding region of the *NtPMT1a* gene was found to be identical to the A411 cDNA (Hibi *et al.*, 1994), with the exception of a single base change in Exon 6 that results in a conservative amino acid substitution. This difference could be the result of minor differences among cultivars used in the two studies (i.e., Xanthi vs. Burley 21). Translation of the open reading frame contained in *NtPMT1a* showed that it encoded a protein containing four N-terminal 11 amino acid repeats, similar to Exon 1 of the *PMT* gene present in *N. tomentosiformis* (Hashimoto *et al.*, 1998a).

Given the observation that *NtPMT1a* encoded a homolog of the *PMT* gene present in *N. tomentosiformis*, the nature and possible evolutionary origin of the remaining *PMT* gene present in the *N. tabacum* genome was brought into question. From our expression studies (described in detail below), we had determined that five distinct *PMT* encoding transcripts were present in the roots of *N. tabacum*, four of which could be accounted for based upon the length of the Exon I coding region in the four *PMT* genes isolated and characterized in our studies described above. The fifth transcript was similar in size to that encoded by *NtPMT1a* and, therefore, was designated *NtPMT1b*. Since the variability in *PMT* gene structure is primarily localized within Exon 1, we used a PCR-based strategy to analyze the *PMT* gene structure and family size in *N. otophora*, the other possible progenitor of *N. tabacum*. As shown in Figure 3, five distinct PCR products were detected in the electrophoretic pattern of amplification products generated from *N. tabacum* genomic DNA using Exon 1 specific primers (Table 1). Consistent with our studies described above and the previous work of Hashimoto *et al.* (1998a), three PCR products were detected in the electrophoretic pattern of amplification products generated from *N. sylvestris* genomic DNA, and a single band was recovered from *N. tomentosiformis* genomic DNA. Amplification of genomic DNA from *N. otophora* using Exon 1 specific primers also yielded only a single band, whose electrophoretic mobility was most similar to that of the *NtPMT1b* derived product.

Analysis of PMT gene intron and flanking sequences

The location of the seven introns within the protein coding region of the five *PMT* genes in *N. tabacum* is identical and appears to be conserved among *PMT* genes from different *Nicotiana* species. There is also little variation in the nucleotide sequences that comprise the Exon-Intron splice junctions in the various *PMT* genes in *N. tabacum* (Table 2). The high degree of nucleotide sequence similarity recognized among *PMT* genes within their coding regions is also present within their introns and immediate 5' and 3' flanking sequences (Table 2 and Figure 4). In general, a greater level of sequence identity is found in the introns of the *NtPMT2*, *NtPMT3*, and *NtPMT4* genes, than in pair-wise comparisons among the introns of the other members of the *N. tabacum* *PMT* gene family. The observed conservation in the intron sequences of the *NtPMT2*, *NtPMT3*, and *NtPMT4* genes is consistent with their origin from the same progenitor species (*N. sylvestris*). One interesting exception occurs within Intron 6, where the length of the intron and the sequence similarity is more conserved between *NtPMT1a* and *NtPMT4*, than between *NtPMT4* and *NtPMT2* or *NtPMT3*.

Approximately 1 kb of nucleotide sequence was determined 5' to the coding regions of the *NtPMT1a*, *NtPMT2*, *NtPMT3*, and *NtPMT4* genes (Figure 4). By comparison to the 5'-untranslated

region (UTR) present in the A411 cDNA, we set the start site for transcription initiation at approximately 57 nucleotides upstream of the MET start codon in *NtPMT1a* and *NtPMT3*, and either 69 or 60 nucleotides upstream in *NtPMT2* and *NtPMT4*. The major distinguishing feature between the 5'-UTRs in the various genes is the presence or absence of a 17 bp sequence in the gene. An appropriately placed TATA-box can be easily recognized 45 bp 5' to the initiation site in all four genes. Within the first 200-250 bp upstream of the TATA box, a high level of sequence conservation is found to exist among the promoter regions in the four genes. After this point, a clear difference can be observed between the *NtPMT1a* promoter and the remaining three genes, and by 400 bp upstream, little similarity can be found among any of the gene family members.

Analyzing the proximal regions of the various *PMT* promoters with various motif scanning software identified several G-box-like sequences (Foster *et al.*, 1994; Kim *et al.*, 1992; Menkens *et al.*, 1995; Staiger *et al.*, 1989; Williams *et al.*, 1992) at various positions among the *PMT* promoters, and a potential metal response element (MRE) (positions -75 to -66; numbering relative to the *NtPMT1a* promoter sequence) in three of the four *PMTs* (Cizewski-Culotta and Hamer, 1989; Thiele, 1992). An unusual 17 nucleotide stretch of guanine occurs at positions -259 to -243 in the *NtPMT1a* gene promoter followed upstream by a purine-rich region (positions -332 to -263). In the *NtPMT3* promoter a 14 bp palindromic sequence (positions -497 to -484) was detected. *PMT* gene expression has been reported to increase in root tissues following treatment with methyl jasmonate (Imanishi *et al.*, 1998). However, none of the sequence motifs reported to confer methyl jasmonate-responsiveness in other plant genes (Mason *et al.*, 1993; Rouster *et al.*, 1997) were detected in the *PMT* promoters.

Comparison of the available nucleotide sequence information from the 3'-flanking regions of the various *PMT* genes in *N. tabacum* revealed that the 3'-UTRs in the *NtPMT2*, *NtPMT3*, and *NtPMT4* genes of *N. tabacum* share approximately 81-94% identity with each other and are essentially identical to those reported for *N. sylvestris* *PMTs* by Hashimoto *et al.* (1998a). The major distinguishing feature among the various genes is the presence of two short (20 bp and 4 bp) deletions in the *NtPMT2* gene, which lowers the percent identity. The 3'-UTR of *NtPMT1a* is identical to that reported for the A411 cDNA (Hibi *et al.*, 1994) and 81-94% identical to the other *PMT* genes in the *N. tabacum* genome. Unfortunately, no sequence information is currently available for the 3'-UTR of the *N. otophora* or *N. tomentosiformis* *PMT* genes.

Regulation of PMT gene expression

To determine whether the members of the *PMT* gene family in *N. tabacum* were differentially

expressed, a series of experiments were carried out to define the temporal and spatial distribution of transcripts arising from the five genes. Shown in Figure 5A are the results of gel blot analysis of total RNA extracted from various tissues of mature Burley 21 tobacco plants hybridized with radioactively-labeled probe capable of detecting all five *PMT* transcripts. Consistent with previous studies (Hashimoto *et al.*, 1998b; Hibi *et al.*, 1994), *PMT* expression is localized exclusively to roots. When maturing wild-type (HP) Burley 21 plants are topped (i.e., the floral meristem and upper 1/3 of the stem are removed), a dramatic increase in *PMT* transcript abundance is observed within 2 hr, reaching a maximal level of accumulation by 12-24 hr. Two size transcripts are detected on the gel blots, reflecting the small difference in message size that occurs as a result of the difference in size of Exon 1 among the genes.

In addition to examining *PMT* gene expression in wild-type plants, we also examined expression in a low nicotine-producing (LP) mutant of Burley 21 (Legg and Collins, 1971). The low nicotine Burley 21 line harbors mutations at two independent loci (*nic1* and *nic2*) thought to be global regulators of gene expression involved in alkaloid formation. As shown in Figure 6B, topping of the low nicotine mutant (*nic1nic2*) Burley 21 did not cause an increase in *PMT* transcript abundance as observed in wild type plants. Thus, it appears that *Nic1* and *Nic2* are likely involved in regulation of *PMT* expression in the very least, and may also be involved in the regulation of other genes in the alkaloid biosynthetic pathway. Whether this is a direct effect (e.g., transcriptional activation) or indirect remains to be determined.

In order to determine the extent to which the individual members of the gene family contributed to the general pattern of expression described above, a semi-quantitative RT-PCR strategy (Kinoshita *et al.*, 1992) was used to detect and quantify the levels of the individual *PMT* transcripts in the roots of both wild-type (HP) and low alkaloid (LP) Burley 21 tobacco. This approach takes advantage of the fact that Exon 1 is variable in length among the various *PMT* genes (Figure 2), allowing for their individual detection and quantitation following polyacrylamide gel electrophoresis and autoradiography.

Five RT-PCR products (representing Exon 1 from each of the five genes present in *N. tabacum*) were detected in the electrophoretic profiles of amplification products derived from reactions using either HP or LP Burley 21 root RNA (Figure 6A). All five *PMT* genes present in the *N. tabacum* genome were expressed in the roots of wild-type plants, and topping resulted in a differential accumulation of transcripts derived from each gene. Among the five genes, transcripts derived from the *NtPMT2* and *NtPMT1b* showed the greatest increase in abundance rising approximately 3-fold during the first 24 hr post-topping, whereas levels of the *NtPMT1a* and *NtPMT4* transcripts changed little in response to topping (Figure 6B). In the LP mutant, little or no effect was observed on the

levels of the various *PMT* transcripts following topping, although in some cases (e.g., *NtPMT1a*) a small but likely insignificant depression in transcript abundance was detected. Thus, it appears that all five genes contribute to *PMT* activity levels within the root.

II. ADC

The present invention features the characterization of two members of the nuclear gene family encoding ADC in tobacco *N. tabacum* L. As the following discussion shows, *ADC2* is preferentially expressed in roots and accounts for the major portion of *ADC* transcripts present. Furthermore, analysis of *ADC* transcript levels in roots of low and high nicotine producing lines showed that *ADC* expression is under the control of the *Nic1 Nic2* regulatory loci.

Materials and methods

Plant growth and tissue preparation

Seeds of *N. tabacum* cv. Xanthi, wild-type and low alkaloid *nic1 nic2* mutant *N. tabacum* cv. Burley 21 were obtained from Dr. G. Collins (University of Kentucky, Lexington). Tobacco plants used for DNA isolation were grown in soil:vermiculite mixture in the greenhouse under natural lighting conditions. Plants used for RNA extraction were grown either in Moltan Plus (Moltan Co., Middleton, TN) or hydroponically in a dilute (half-strength) Peters nutrient solution with continuous aeration of the roots under natural lighting conditions in the greenhouse. Topping experiments were conducted by removing the floral meristem, leaves and stem (approximately the upper 1/3 of the plant) from tobacco plants just prior to blooming. Plant tissues were collected from fully matured individuals, frozen in liquid nitrogen, and stored at -80°C until used for RNA preparation (see below).

Screening of genomic libraries and phage characterization

A genomic library constructed in λ EMBL3 from *N. tabacum* cv. Xanthi leaf DNA (Clontech, Inc., Palo Alto, CA) was screened by plaque hybridization (Sambrook *et al.*, 1989) using an [α -³²P]-dCTP-labeled, 2.7 kb *EcoRI-XhoI* fragment from plasmid PR24 as probe. PR24 encodes a full length ADC cDNA isolated from the roots of wild-type *N. tabacum* cv. Burley 21 (Wang, 1999). Hybridization was performed at 65°C for 16 h in a solution containing 0.25 M Na₂HPO₄ (pH 7.2) and 7% (w/v) SDS. Following hybridization, the membranes were washed twice in 2 x SSC, 0.1%

SDS for 15 min at room temperature, once in 0.2 x SSC, 0.1% SDS for 30 min at 65°C. Hybridizing phage were picked and plaque purified through three subsequent rounds of hybridization screening. Phage DNA was isolated from plaque purified phage using a Qiagen Phage Midi Preparation Kit (Qiagen, Germany) and insert DNA characterized by restriction mapping and DNA gel blot analysis.

- 5 The relevant hybridizing bands in each phage were cloned into pBluescript SK+ vectors for further analysis.

Nucleic acid sequencing and analysis

- 10 Nucleotide sequencing was carried out manually using the Sequenase Version 2.0 protocols according to the manufacturer's protocol (United States Biochemical, Cleveland, OH) or with an ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) using double-stranded plasmid DNA templates prepared utilizing the Qiaprep Spin Plasmid Kit (Qiagen USA, Valencia, CA). The nucleotide and predicted amino acid sequences of the various cDNAs were analyzed using BLAST
15 sequence analysis programs (Altschul *et al.*, 1990; Gish and States, 1993) and protein sequence alignments were carried out using the PILEUP program (Genetics Computer Group Sequence Analysis package, Version 9.0 (GCG, University of Wisconsin, Madison, WI) and the various gene sequences available in the NCBI (National Center for Biotechnology Information, Bethesda, MD) nucleotide and protein sequence database. Manual adjustment of the sequence alignments were
20 carried out as necessary.

RNA isolation and gel blot analysis

- Total RNA was extracted from tobacco roots, leaves, and floral parts using Tri-Reagent
25 (Molecular Research Center, USA, Cincinnati, OH) according to the manufacturer's protocol. For RNA gel blot analysis, aliquots (10 µg) of total RNA extracted from the various tissues were fractionated by electrophoresis through a 1.2% agarose-formaldehyde gel and blotted onto Nytran nylon membranes (Schleicher & Schuell, Keene, NH) using 10 X SSC. The transferred RNA was UV cross-linked to the membrane using a UV Stratalinker (Stratagene, La Jolla, CA) and the
30 membranes were prehybridized in 7% SDS, 0.25 M Na₂HPO₄, pH 7.2 for 2-4 hours at 65°C. Hybridization was carried out in the same buffer in the presence of ³²P-labeled probes for 16 hr at 65°C. The membranes were washed under high stringency conditions and subject to autoradiography at -80°C for approximately 48 h.

For gel blot analysis, [α- ³²P]-dCTP -labeled probes were prepared by random primed labeling

(Random Primed Labeling Kit, Boehringer Mannheim, Indianapolis, IN) using 25-50 ng of a 2.7 kb *EcoRI-XhoI* fragment derived from PR24 and a 460 bp fragment amplified from the β - subunit of the tobacco mitochondrial ATP synthase gene (*atp2*) (Boutry and Chua, 1985).

5 *Semi-quantitative RT-PCR analysis of NtADC1 and NtADC2 transcript levels.*

Total RNA (2 μ g) from roots, leaves, or floral parts was reverse transcribe at 40°C for 1 h in a reaction cocktail containing 200 units of SuperscriptII reverse transcriptase (RNase H-, Gibco BRL, USA), 10 units RNase inhibitor (Perkin Elmer), 200 μ M dNTPs and 40 pmol of primer, in total
10 volume of 20 μ l. For first strand cDNA synthesis, a single primer [5'-AGAAAAACATCACCAACT-3'] capable of hybridizing to both the *ADC1* and *ADC2* transcripts was used in the reaction. As a control, a primer (5'-GCAACTGTCATCTTATCATCTTC-3') specific for the β -subunit of the tobacco mitochondrial ATP synthase gene *atp2* (Boutry and Chua, 1985) was used in the reverse transcriptase reaction.

15 Following reverse transcription, the single stranded cDNA products were serially diluted over a concentration range between 1 to 50 ng RNA, and PCR amplification was carried out for 25 cycles of 45 s at 94°C, 1 min at 64°C and 1 min at 72°C in a Genemate thermocycler (ISC Bioexpress, UT). The reaction mixture contained cDNA template, 1 x PCR buffer (Boehringer Mannheim), 100 μ M dNTPs, 25 pmol of each forward and reverse primer and 1 unit Taq DNA polymerase. The PCR
20 reactions specific for *ADC1* transcripts contained the following primers: ADC1-forward, 5'-CGTAGACGCTACTGTTTC-3' and ADC1-reverse, 5'-TGGACAAC TGTGGAGGCG-3'. Reactions specific for *ADC2* transcripts contained primers ADC2-forward, 5'-TG TAGATGCTGCTGTTGTTT-3', and ADC2-reverse, 5'-TGAACAAC TGCGGAGGCA-3'. Control reactions for normalization of amplification products contained 25 pmol of primers specific
25 for the tobacco *atp2* transcripts: *atp2* forward, 5'-GTATATGGTCAAATGAATGAGCC-3', and *atp2* reverse.int, 5'-GCAGTATTGTAGTGATCCTCTCC-3'. For quantitation purposes, amplification reactions were supplemented with 1 μ Ci ³²P-dCTP. PCR products were separated by electrophoresis through 1.2% agarose gels, the fractionated reaction products transferred onto a Hybond N+ membranes, dried and subject to autoradiography at -70° C. Quantitation was carried out by
30 phosphorimaging using a Molecular Dynamics PhosphorImager. Values were normalized relative to the intensities of the *atp2* control band in each lane. The experiment was conducted twice with different total RNA samples, and representative results are presented from one of the two experiments.

Results

These studies show the structure and expression of individual members of the *ADC* gene family in tobacco. An α -³²P-dCTP-labeled 2.7 kb EcoRI-XhoI fragment from PR24 encoding the ADC coding region was used to screen an λ EMBL3 phage genomic library. From a screen of approximately 3 X10⁵ phage, seventeen hybridizing phage were recovered, of which five were fully characterized by restriction mapping and DNA gel blot analysis. These phage fell into two groups based on their restriction profile. The relevant hybridizing fragments from the various phage were cloned into pBluescript and their nucleotide sequence determined.

Presented in Figure 7 are the nucleotide and predicted amino acid sequences of NtADC-1 and NtADC-2 genes. Both genes contain a single open reading frame, uninterrupted by introns. The nucleotide and amino acid sequence encoded in NtADC-1 is identical to that of PR24, the full length cDNA isolated from *N. tabacum* cv Burley 21. There are 84 nucleotide differences within the NtADC-1 and NtADC-2 coding regions, resulting in 23 amino acid differences between the ADC1 and ADC2 proteins, respectively. The ADC1 protein is one amino acid shorter in length, missing Val-13.

By comparison to the full-length cDNA, the 5'-untranslated region (UTR) present in NtADC-1 and NtADC-2 are 431 bp and 432 bp long, respectively. The size of the 5'-UTR in the ADC transcripts is considerably larger than the average size of the plant leader sequence (Joshi, 1987). In contrast, the 3'-UTRs present in NtADC-1 and NtADC-2 are relatively short, approximately 84 nucleotides in length. In both gene sequences, a conserved polyadenylation signal (AATAATA) can be recognized 23 nucleotides from the site of polyadenylation site found in the PR24 cDNA.

Pairwise comparison of the *N. tabacum* ADC1 and ADC2 proteins with the ADCs of other plant species showed that the *N. tabacum* proteins are approximately 82% identical to the ADC of its evolutionary progenitor species *N. sylvestris* [Genbank Accession No. AB012873] and 86% identical to the ADC from tomato (*Lycopersicon esculentum*) [31], another member of the Solanaceae family (Figure 2). As might be expected, the *N. tabacum* ADC shares considerably less similarity to ADCs isolated from species more distantly related evolutionarily, such as *Arabidopsis* - 67% identical [32, 33], soybean- 67% identical [34], and oat - 42% identical [35] and is only 29% identical to the enzyme from *Escherichia coli* - [36].

The predicted protein coding regions for the *N. tabacum* ADCs are substantially longer than those reported for the ADC proteins of *N. sylvestris* and *L. esculentum* [31], but are similar in length to those reported in *Arabidopsis*, oat, soybean [32-35] and for the *E. coli* enzyme [36]. The

difference in overall length appears to arise from an apparent nucleotide deletion in the *N. sylvestris* and tomato cDNA sequences relative to the ADC1 and ADC2 predicted sequence and those in other plants. In the nucleotide sequences reported for both the *N. sylvestris* and tomato cDNAs, a guanine residue (position 2295 in the *N. sylvestris* sequence and 1531 in the tomato sequence) is missing [Genbank Accession No. AB012873]. This deletion changes the reading frame and introduces a premature termination to the predicted coding region. Using the sequence information available in the NCBI database, correcting for this error allowed us to extend the predicted C-terminus of the both ADC proteins, yielding the alignment to the *N. tabacum* ADCs and those of other plant ADCs as indicated in Figure 8. We have also included in the alignment shown in Figure 8, the correction at the N-terminus of the predicted tomato ADC protein sequence noted by Pérez-Amado et al. [37], allowing better alignment of all of the higher plant sequences.

Developmental regulation of arginine decarboxylase expression

It has been shown that nicotine formation can be activated in the roots of maturing tobacco plants by topping, that is, removal of the flower head and several young leaves (Akehurst, 1981; Hibi, et al., 1994). Coincident with the activation of nicotine formation, there is an increase in the levels of transcripts encoding ODC, PMT and spermidine synthase (SPS) over the subsequent 24 hr period in wild-type plants (Hibi et al., 1994; Riechers and Timko, 1999). To determine the effects of topping on ADC expression in roots, Burley 21 plants were grown in the greenhouse to the bud stage at which point the upper 1/3 of the plant was removed and samples of roots tissues were collected before and at various times post-topping. As shown in Figure 9, ADC message abundance increased in the roots of topped Burley 21 plants during the 24 hr period after topping. Low alkaloid (LA) mutants of Burley 21 show a much lower level of ADC expression in their roots, and no induction of ADC transcript accumulation after topping. The lack of ADC induction in the low-alkaloid mutant is consistent with previous studies (Hibi et al., 1994; Riechers and Timko, 1999; Wang, 1999) showing a general inability to activate gene expression leading to increased polyamine formation and alkaloid biosynthesis as a result of the mutation of the *Nic1* and *Nic2* regulatory genes.

NtADC-2 is predominately expressed in roots of wild-type plants.

Due to the high degree of identity between the NtADC-1 and NtADC-2 transcripts (e.g., 95.8% coding regions, 94.4% and 96.4% in 5'- and 3'-UTRs, respectively), it is impossible to distinguish between the two transcripts by RNA gel bot analysis. Therefore, we employed a RT-PCR based

strategy and gene specific oligonucleotide primers. Total RNA was extracted from tobacco roots, leaves and flowers, and single-stranded cDNA synthesized using an oligonucleotide primer capable of hybridizing to both ADC1 and ADC2 transcripts. As an internal control for amplification, a gene specific primer recognizing the *atp2* transcript encoding the β -subunit of the tobacco mitochondrial ATPase was include in the reactions. Under experimental conditions providing amplification in the linear range of the PCR reaction, gene specific forward and reverse primers were used to specifically amplify either ADC1 or ADC2 cDNAs. Test reactions (Figure 10A) using plasmid DNA encoding NtADC1 or NtADC2 as template demonstrated the specificity of the primers. As shown in Figure 10B, the main transcripts detectable in all tissues tested are derived from NtADC-2. Flowers express the highest level of ADC, and leaves lowest. In the flowers, although ADC1 is detectable, far less than ADC2. Roots also express a significant level of ADC.

ADC transcript levels are highest in the roots and floral organs, and low in other plant tissues. The two ADC genes investigated appear to have different modes of regulation, with ADC2 being predominately expressed in the roots and other organs.

At the present time, only limited information is available on the nature of regulatory regions in the promoters of genes encoding enzymes of alkaloid biosynthesis. The availability of cloned genomic fragments encoding ADC allows one to begin mapping regulatory sequences within members of these genes responsible for tissue specific, developmental, and inducible expression.

III. ODC

The present invention features the genes of two members of the nuclear gene family encoding ODC in tobacco *N. tabacum*. As the following experimental discussion shows, the ODC-2 gene is preferentially expressed in roots and floral tissues. Furthermore, the abundance of ODC transcripts in root tissues is affected by topping. Furthermore, analysis of ODC transcript levels in roots of low and high nicotine producing lines shows that ODC expression is under the control of the *Nic1* *Nic2* regulatory loci.

Materials and methods

Plant growth and tissue preparation

Seeds of *N. tabacum* cv. Xanthi, wild-type and low alkaloid *nic1 nic2* mutant *N. tabacum* cv. Burley 21 were obtained from Dr. G. Collins (University of Kentucky, Lexington). Tobacco plants used for DNA isolation were grown in soil:vermiculite mixture in the greenhouse under natural lighting

conditions. Plants used for RNA extraction were grown either in Moltan Plus (Moltan Co., Middleton, TN) or hydroponically in a dilute (half-strength) Peters nutrient solution with continuous aeration of the roots under natural lighting conditions in the greenhouse. Topping experiments were conducted by removing the floral meristem, leaves and stem (approximately the upper 1/3 of the plant) from tobacco plants just prior to blooming. Floral parts and other plant tissues were collected from fully matured individuals, frozen in liquid nitrogen, and stored at -80°C until used for RNA preparation (see below).

Screening of genomic libraries and phage characterization

A genomic library constructed in EMBL3 from *N. tabacum* cv. Xanthi leaf DNA (Clontech, Inc., Palo Alto, CA) was screened by plaque hybridization (Sambrook *et al.*, 1989) using a ³²P-radiolabeled, 1.6 kb *EcoRI-XhoI* insert from plasmid PR46 as probe. PR46 encodes a full length ODC cDNA previously isolated by differential screening of plasmid libraries prepared from mRNA isolated from the roots of wild-type Burley 21 plants before and 3-days post-topping (Wang, J., Sheehan, M., Bookman, H. and Timko, M.P., unpublished data). Hybridization was performed at 65°C for 16 h in a solution containing 0.25 M Na₂HPO₄ (pH 7.2) and 7% (w/v) SDS. Following hybridization, the membranes were washed twice in 2 x SSC, 0.1% SDS for 15 min at room temperature, once in 0.2 x SSC, 0.1% SDS for 30 min at 65°C. Hybridizing phage were picked and plaque purified through three subsequent rounds of hybridization screening. Phage DNA was isolated from plaque purified phage using a Qiagen Phage Midi Preparation Kit (Qiagen USA, Valencia, CA) and insert DNA characterized by restriction mapping and DNA gel blot analysis. The relevant hybridizing bands in each phage were cloned into pBluescript SK+ vectors for further analysis.

Nucleic acid sequencing and analysis

Nucleotide sequencing was carried out manually using the Sequenase Version 2.0 protocols according to the manufacturer's protocol (United States Biochemical, Cleveland, OH) or with an ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) using double-stranded plasmid DNA templates prepared utilizing the Qiaprep Spin Plasmid Kit (Qiagen USA, Valencia, CA). The nucleotide and predicted amino acid sequences of the various cDNAs were analyzed using BLAST sequence analysis programs (Altschul *et al.*, 1990; Gish and States, 1993) and protein sequence alignments were carried out using the PILEUP program (Genetics Computer Group Sequence Analysis package, Version 9.0 (GCG, University of Wisconsin, Madison, WI) and the various gene sequences available in the NCBI (National Center for Biotechnology Information, Bethesda, MD) nucleotide and protein sequence database. Manual adjustment of the sequence alignments were

carried out as necessary.

RNA isolation and gel blot analysis

Total RNA was extracted from tobacco roots, leaves, and floral parts using Tri-Reagent (Molecular Research Center, USA, Cincinnati, OH) according to the manufacturer's protocol. For RNA gel blot analysis, aliquots (10 µg) of total RNA extracted from the various tissues were fractionated by electrophoresis through a 1.2% agarose-formaldehyde gel and blotted onto Nytran nylon membranes (Schleicher & Schuell, Keene, NH) using 10 X SSC. The transferred RNA was UV cross-linked to the membrane using a UV Stratalinker (Stratagene, La Jolla, CA) and the membranes were prehybridized in 7% SDS, 0.25 M Na₂HPO₄, pH 7.2 for 2-4 hours at 65°C. Hybridization was carried out in the same buffer in the presence of ³²P-labeled probes for 16 hr at 65°C. The membranes were washed under high stringency conditions and subject to autoradiography at - 80°C for approximately 48 h.

Restriction fragments derived from cDNA clones of interest were separated by agarose gel electrophoresis, the DNA was purified, and quantified by spectrophotometry. [³²P]-dCTP -labeled probes were prepared from 25-50 ng of insert DNA by random primed labeling (Random Primed Labeling Kit, Boehringer Mannheim, Indianapolis, IN). As a control, the blots were also probed with radioactively labeled probes encoding the alkaloid biosynthesis enzyme putrescine N-methyltransferase (PMT) (Riechers and Timko, 1999), a root specific, topping inducible β-glucosidase encoding cDNA (TBG-1) (Riechers, D.E. and Timko, M.P., unpublished data), 26S rRNA (PR31) or 28S rRNA fragments.

Genomic DNA isolation and gel blot analysis

Tobacco genomic DNA was prepared from tobacco leaf tissue by the method of Junghans and Metzlaff (1990). Total genomic DNA (15 µg) was digested to completion with *Eco*RI or *Hind*III, the digestion products were fractionated by electrophoresis through a 0.8% (w/v) agarose gel, and transferred onto Nytran nylon membrane (Schleicher & Schuell, Keene, NH) in the presence of 0.4 N NaOH (Sambrook *et al.*, 1989). Following transfer, the membrane was rinsed in 2 X SSC, the DNA was UV cross-linked to the membrane, and the membrane was prehybridized and hybridized as described above. Following hybridization and washing, the membranes were subjected to autoradiography at -80°C.

Results and discussion

Gel blot analysis of tobacco genomic DNA cut with various restriction enzymes and hybridized with an [α - 32 P]-dCTP-labeled 1.6 kb *EcoRI-XhoI* cDNA fragment (PR46) encoding the full-length ODC protein from *N. tabacum* cv Burley 21 (Wang, J., Sheehan, M., Bookman, H. and Timko, M.P., unpublished data) indicated ODC is encoded by small gene family in the *N. tabacum* genome (Fig. 11). Four to five major bands and several minor bands of sufficient size to encode full-length genes are detected in either *EcoRI* or *HindIII* digested tobacco DNA.

To further analyze the structure and regulation of members of the ODC gene family in tobacco, a λ EMBL3 phage genomic library constructed with DNA from *N. tabacum* cv Xanthi was screened using a [α - 32 P]-labeled probes prepared from PR46 (as described above). From a screen of approximately 3×10^5 phage, five hybridizing phage were recovered, of which three were fully characterized by restriction mapping and DNA gel blot analysis. Two phage proved to contain identical insert DNA and the third had a unique restriction digestion profile. Following DNA gel blot analysis, the hybridizing fragments were cloned into pBluescript and their nucleotide sequence determined.

The complete *NiODC-2* gene spans two *SalI* fragments of 2.7 kb and 6.5 kb. The coding region of the gene contains a single 1302 bp open reading frame uninterrupted by introns (Fig. 12). The nucleotide sequences of *NiODC-2* is identical within the coding and 5' and 3'- untranslated regions to the PR46 encoded cDNA, with the exception of four nucleotide changes (residues +2, +4, +6 and +8) in the 5'-untranslated region. These nucleotide differences likely reflect changes introduced during the cDNA synthesis reaction.

The predicted amino acid sequence for the *NiODC-2* encoded protein (designated pODC2) (Fig. 13) is identical to the ODC characterized from Burley 21 tobacco encoded by PR46 (Wang, J., Sheehan, M., Bookman, H. and Timko, M.P., unpublished data) and to the partial *N. tabacum* ODC cDNA sequence (PR17) reported by Malik *et al.*, (1996). Comparison of the predicted amino acid sequence for pODC2 with the ODC proteins characterized from two different tobacco cultivars showed that the pODC2 differs by 7 amino acid (98% identity) from the ODC protein characterized from the high alkaloid cultivar, *N. tabacum* cv. SC58 [Genbank Accession No. Y10472.1] and by 7 amino acid (98% identity) from ODC protein from BY-2 cells. The tobacco pODC2 is 89% and 90% identical to the ODCs from tomato (*Lycopersicon esculentum*) and jimsonweed (*Datura stramonium*), respectively, but substantially less similar to ODCs from yeast (35% identity) and humans (32% identity).

The *NiODC-1* gene, contained on an 4.0 kb *XbaI* fragment, encodes a single open reading frame of 141 amino acids encompassing the amino terminal one-half of ODC (Fig. 12). Six amino acid residue changes distinguish the *NiODC-2* and *NiODC-1* encoded proteins over the homologous

region of the proteins. Beginning at amino acid residue 130, the *NtODC-1* encoded protein (pODC1) diverges from pODC2, with a stop codon present after residue 141. Scanning the available nucleotide sequence (> 1 kb) in the 3'-flanking region of the *NtODC-1* gene failed to reveal any evidence for ODC homologous protein sequences in any of the three translational reading frames.

5 Interestingly, a comparison of the 5'-flanking sequence of the *NtODC-1* and *NtODC-2* genes revealed that while the *NtODC-2* gene has a clearly recognizable TATA-box properly located at approximately -35 bp from the transcriptional start site, no such regulatory motif is found in the *NtODC-1* gene sequence. Consistent with this observation, RNA gel blot analysis performed using a hybridization probe prepared from *NtODC-1* immediately downstream of the frame shift, failed to
10 detect any message in various tissues of mature tobacco plants (data not shown). Thus, it appears that *NtODC-2* represents an unexpressed pseudogene in the *N. tabacum* genome.

To determine the spatial pattern of expression of the *NtODC-2* gene, gel blot analysis was carried out using total RNA prepared from roots, stems, young and mature leaves, and various floral parts of Burley 21 tobacco plants. As shown in Fig 14, transcripts encoding ODC were easily
15 detected in the roots, with little or no expression in other tissues except sepals, carpels, and mature stamens.

The formation of nicotine and total leaf alkaloids in tobacco is known to be under the control of at least two independent genetic loci (Legg *et al.*, 1969; Legg and Collins, 1971), designated *Nic1* and *Nic2* (Hibi *et al.*, 1994). *Nic1* and *Nic2* are semidominant and operate synergistically to control
20 plant alkaloid content, with mutations within these genes resulting in plants with reduced levels of nicotine and total leaf alkaloids (wild-type > *nic1* > *nic2* > *nic1 nic2*) (Legg *et al.*, 1969; Legg and Collins, 1971). Although no information is available on the nature of their encoded products, it has been speculated that *Nic1* and *Nic2* likely encode transcriptional regulators capable of globally interacting with a subset of genes encoding components of polyamine and alkaloid biosynthesis
25 (Hibi *et al.*, 1994). Removal of the flower head and several young leaves (i.e., topping) leads to activation of nicotine formation in the roots of decapitated plants (Akehurst, 1981; Hibi *et al.*, 1994). To determine the effects of topping on *NtODC-1* expression in roots, Burley 21 plants were grown in the greenhouse to the bud stage at which point the upper 1/3 of the plant was removed and samples of roots tissues were collected before and at various times post-topping. As shown in Fig 14B, low
30 levels of the *ODC* transcripts were found in roots prior to topping and message abundance increased approximately 2-fold in the roots of topped Burley 21 plants 4 hr after topping. By 24 hr after topping, *ODC* transcript levels return to their initial levels. Low alkaloid mutants of Burley 21 subjected to the same treatment show a much lower level of stimulation of *ODC* transcript accumulation after topping, and the enhanced transcript abundance does not persist beyond 4 hr. By

comparison, transcripts encoding PMT and a tobacco root-specific β -glucosidase (TBG-1) show patterns of accumulation similar to that observed for ODC transcripts in wild-type plants, but no induction in the low-alkaloid mutant, consistent with previous studies (Hibi *et al.*, 1994; Riechers and Timko, 1999; Wang, 1999).

IV. SAMS

A single recombinant phage is identified as encoding for SAMS. This λ phage contains an approximately 15kB SalI insert. Restriction mapping and PCR analysis indicates that the insert DNA contains primarily the coding and 3'non-coding portions of the SAMS gene. The nucleotide sequences for the gene encoding SAMS can be found at GenBank Accession Nos. AF27243 (full length SAMS cDNA).

V. NADH dehydrogenase

A fragment of the cDNA encoding for NADH dehydrogenase in *N. tabacum* shows high expression in the roots of mature wild-type HP plants compared to low alkaloid mutant LP plants.

VI. Phosphoribosylanthranilate isomerase (PAI)

The gene encoding for a fragment of phosphoribosylanthranilate isomerase in *N. tabacum* is a homolog of the *Arabidopsis thaliana* gene encoding PAI, an enzyme involved in tryptophan biosynthesis. This enzyme is involved in the overall formation of aromatic compounds in plants.

REFERENCES

Akehurst BC. 1981. The growth, plant structure and genetics. In: Rhind D, Wrigley G, eds., Tobacco, London: Longman Press, 45-95.

Alabadi D, Carbonell J. 1998. Expression of ornithine decarboxylase is transiently increased by pollination, 2,4-dichlorophenoxyacetic acid, and gibberellic acid in tomato ovaries. *Plant Physiology* 118: 323-328.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic alignment search tool. *Journal of Molecular Biology* 215: 403-410.

Baldwin IT. 1989. Mechanism of damage-induced alkaloid production in wild tobacco. *Journal of Chemical Ecology* 15: 1661-1680.

Baldwin IT, Prestin CA. 1999. The eco-physiological complexity of plant responses to insect herbivores. *Planta* **208**:137-145.

Baldwin IT, Schmelz EA, Ohnmeiss TE. 1994. Wound-induced changes in root and shoot
5 jasmonic acid pools correlate with induced nicotine synthesis in *Nicotiana sylvestris* Spegazzini and Comes. *Journal of Chemical Ecology* **20**: 2139-2157.

Baldwin IT, Schmelz EA, Zhang Z-P. 1996. Effects of octadecanoic metabolites and inhibitors on
induced nicotine accumulation in *Nicotiana sylvestris*. *Journal of Chemical Ecology* **22**: 61-74.

10 **Baldwin IT, Zhang Z-P, Diab N, Ohnmeiss TE, McCloud ES, Lynds GY, Schmelz EA.** 1997. Quantification, correlations, and manipulations of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*. *Planta* **201**: 397-404.

15 **Bell E. and R.L. Malmberg,** Analysis of a cDNA encoding arginine decarboxylase from oat reveals similarity to the *Escherichia coli* arginine decarboxylase and evidence of protein processing. *Mol. Gen. Genet.*, **224** (1990) 431-436.

Boutry M. and N.H. Chua, A nuclear gene encoding the beta subunit of the mitochondrial ATP
20 synthase in *Nicotiana plumbaginifolia*. *EMBO J.*, **4** (1985) 2159-2165.

Bracher D, Kutchan TM. 1992. Strictosidine synthase from *Rauvolfia serpentina*: analysis of a gene involved in indole alkaloid biosynthesis. *Archives of Biochemistry and Biophysics* **294**: 717-723.

25 **Chattopadhyay MK, Ghosh B.** 1998. Molecular analysis of polyamine biosynthesis in higher plants. *Current Science* **74**, 517-522.

Chou W-M, Kutchan TM. 1998. Enzymatic oxidations in the biosynthesis of complex alkaloids. *Plant Journal* **15**, 289-300.

30 **De Luca V. and B. St. Pierre.** 2000. The cell and developmental biology of alkaloid biosynthesis. *Trends in Plant Science* **5**: 168-173.

Eilbert U. 1998. Induction of alkaloid biosynthesis and accumulation in plants and *in vitro* cultures

in response to elicitation. In: Roberts MF, Wink M, eds. Alkaloids: Biochemistry, Ecology, and Medicinal Applications. New York: Plenum Press, 219-262.

5 **Facchini PJ, Penzes-Yost C, Samanani N, Kowalchuk B.** 1998. Expression patterns conferred by tyrosine/dihydroxyphenylalanine decarboxylase promoters from opium poppy are conserved in transgenic tobacco. *Plant Physiology* 118: 69-81.

Galloway G.L., R.L. Malmberg and R.A. Price, Phylogenetic utility of the nuclear gene arginine decarboxylase: an example from Brassicaceae. *Molec. Biol. & Evol.*, 15 (1998) 1312-1320.

10 **Gantet P, Imbault N, Thiersoult M, Doireau P.** 1998. Necessity of a functional octadecanoic pathway for indole alkaloid synthesis by *Catharanthus roseus* cell suspensions cultured in an auxin-starved medium. *Plant & Cell Physiology* 39: 220-225.

15 **Gish W, States DJ.** 1993. Identification of protein coding regions by database similarity search. *Nature (Genetics)* 3: 266-272.

Goddijn OJM, de Kam RJ, Zanetti A, Schilperoort A, Hoge JHC. 1992. Auxin rapidly down regulates transcription of the tryptophan decarboxylase gene from *Catharanthus roseus*. *Plant*
20 *Molecular Biology* 18: 1113-1120.

Hashimoto T, Yamada Y. 1994. Alkaloid biogenesis: molecular aspects. *Annual Review of Plant Physiology and Plant Molecular Biology* 45, 257-285.

25 **Hashimoto T, Shoji T, Mihara T, Oguri H, Tamaki K, Suzuki K-i, Yamada Y.** 1998. Intraspecific variability of the tandem repeats in *Nicotiana* putrescine N-methyltransferases. *Plant Molecular Biology* 37: 25-37.

Hibi N, Higashiguchi S, Hashimoto T, Yamada Y. 1994. Gene expression in tobacco low-nicotine
30 mutants. *Plant Cell* 6: 723-735.

Imanishi S, Hashizume K, Nakakita M, Kojima H, Matsubayashi Y, Hashimoto T, Sakagami Y, Yamada Y, Nakamura K. 1998a. Differential induction by methyl jasmonate of genes encoding ornithine decarboxylase and other enzymes involved in nicotine biosynthesis in tobacco cell cultures.

Plant Molecular Biology 38: 1101-1111.

Imanishi S, Hashizume K, Kojima H, Ichihara A, Nakamura K. 1998b. An mRNA of tobacco cell, which is rapidly inducible by methyl jasmonate in the presence of cycloheximide, codes for a putative glycosyltransferase. *Plant & Cell Physiology* 39: 202-211.

Junghans H, Metzlaff M. 1990. A simple and rapid method for preparation of total plant DNA. *Biotechniques* 8: 176.

Kanegae T, Kajiya H, Amano Y, Hashimoto T, Yamada Y. 1994. Species-dependent expression of the hyoscyamine 6 β -hydroxylase gene in the pericycle. *Plant Physiology* 105: 483-490.

Kutchan TM. 1995. Alkaloid biosynthesis - the basis for metabolic engineering of medicinal plants. *Plant Cell* 7, 1059-1070.

Kutchan TM. 1998. Molecular genetics of plant alkaloid biosynthesis. In: Cordell GA, ed., *The Alkaloids, Chemistry and Biology*. San Diego: Academic Press, 295-304.

Legg PD, Collins GB. 1971. Inheritance of percent total alkaloid in *Nicotiana tabacum* L. II Genetic effect of two loci in Burley 21 X LA Burley 21 populations. *Canadian Journal of Genetics and Cytology* 13: 287-291.

Legg PD, Chaplin JF, Collins GB. 1969. Inheritance of percent total alkaloids in *Nicotiana tabacum* L. *Journal of Heredity* 60: 213-217.

Lopes Cardosa MI, Meijer AH, Rueb S, Queiroz Machado J, Memelink J, Hoge JHC. 1997. A promoter region that controls basal and elicitor-inducible expression levels of NADPH: cytochrome P450 reductase (*Cpr*) from *Catharanthus roseus* binds nuclear factor GT-1. *Molecular & General Genetics* 25: 674-681.

Malik V, Watson MB, Malmberg RL. 1996. A tobacco ornithine decarboxylase partial cDNA clone. *Journal of Plant Biochemistry & Biotechnology* 5:109-112.

Malmberg RL, Watson MB, Galloway GL, Yu W. 1998. Molecular genetic analysis of plant

polyamines. *Critical Reviews in Plant Sciences* 17: 199-224.

Michael AJ, Furze JM, Rhodes MJC, Burtin D. 1996. Molecular cloning and functional identification of a plant ornithine decarboxylase. *Biochemical Journal* 314: 241-248.

5

Mizusaki S, Tanabe Y, Noguchi M, Tamaki E. 1973. Changes in the activities of ornithine decarboxylase, putrescine *N*-methyltransferase and *N*-methyl-putrescine oxidase in tobacco roots in relation to nicotine biosynthesis. *Plant & Cell Physiology* 14: 103-110.

10

Nam K.H. , S.H., Lee and J.H. Lee, A cDNA encoding arginine decarboxylase (GenBank U35367) from soybean hypocotyls. *Plant Physiol.*, 110: (1997) 714.

Nam K.H. , S.H. Lee and J.H. Lee, Differential expression of ADC mRNA during development and upon acid stress in soybean (*Glycine max*) hypocotyls. *Plant Cell Physiol.* 38 (1997) 1156-1166.

15

Ohnmeiss TE, McCloud ES, Lynds GY, Baldwin IT. 1997. Within-plant relationships among wounding, jasmonic acid, and nicotine: implications for defense in *Nicotiana sylvestris*. *New Phytologist* 137: 441-452.

20

Pasquali G, Goddijn OJM, de Waal A, Verpoorte R, Schilperoort RA, Hoge JHC, Memelink J. 1992. Coordinated regulation of two indole alkaloid biosynthetic genes from *Catharanthus roseus* by auxin and elicitors. *Plant Molecular Biology* 18: 1121-1131.

25

Pérez-Amador MA, Carbonell J. 1995. Arginine decarboxylase and putrescine oxidase in *Pisum sativum* L. Changes during ovary senescence and early stages of fruit development. *Plant Physiology* 107: 865-872.

30

Pérez-Amador MA, Carbonell J, Granell A. 1995. Expression of arginine decarboxylase is induced during early fruit development and in young tissues of *Pisum sativum* L. *Plant Molecular Biology* 28: 997-1009.

Primikiris, N.I. and K.A. Roubelakis-Angelakis. 1999. Cloning and expression of an arginine decarboxylase cDNA from *Vitis vinifera* L. cell-suspension cultures. *Planta* 208:574-582.

- Riechers DE, Timko MP.** 1999. Structure and expression of the gene family encoding putrescine *N*-methyltransferase in *Nicotiana tabacum*: new clues to the evolutionary origin of cultivated tobacco. *Plant Molecular Biology* 41: 387-401.
- 5 **Rostogi R., J. Dulson and S.J. Rothstein,** Cloning of tomato (*Lycopersicon esculentum* Mill.) arginine decarboxylase gene and its expression during fruit ripening. *Plant Physiol.*, 103 (1993) 829-834.
- Saito K, Murakoshi I.** 1998 Genes in alkaloid metabolism. In: Roberts MF, Wink M, eds.
- 10 *Alkaloids: Biochemistry, Ecology, and Medicinal Applications*. New York: Plenum Press, 147-157.
- Sambrook J, Fritsch EF, Maniatis T.** 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
- 15 **Soyka S and A.G. Heyer.** 1999. *Arabidopsis* knockout mutation of *ADC2* gene reveals inducibility by osmotic stress. *FEBS Lett* 458: 219-223.
- Stim K.P. and G.N. Bennett,** Nucleotide sequence of the *adi* gene, which encoded the biodegradative acid-induced arginine decarboxylase of *Escherichia coli*. *J. Bact.*, 175 (1993) 1221-
- 20 1234.
- Suzuki K, Yamada Y, Hashimoto T.** 1999. Expression of *Atropa belladonna* putrescine *N*-methyltransferase gene in root pericycle. *Plant & Cell Physiology* 40: 289-297.
- 25 **Wang J.** 1999. Characterization of a cDNA (NtADC1) and two nuclear genes (NtgADC1 and NtgADC2) encoding arginine decarboxylase, a key enzyme in alkaloid and polyamine biosynthesis in tobacco (*Nicotiana tabacum* L.). *M.S. Thesis, University of Virginia, Charlottesville, VA*.
- Wang J, Sheehan M, Brookman H, and Timko MP.** 2000. Characterization of cDNAs
- 30 Differentially Expressed in Roots of Tobacco (*Nicotiana tabacum* cv Burley 21) During the Early Stages of Alkaloid Biosynthesis. *Plant Science* In press
- Watson M.B. and R.L. Malmberg,** Regulation of *Arabidopsis thaliana* (L.) Heynh arginine decarboxylase by potassium deficiency stress. *Plant Physiol.*, 111 (1996) 1077-1083.

Watson M.B., W. Yu, G. Galloway and R.L. Malmberg, Isolation and characterization of a second arginine decarboxylase cDNA from *Arabidopsis* (Accession No. AF009647 (PGR97-114). Plant Physiol., 114 (1997) 1569.

5

Watson M.B., K. K. Emory, R.M. Piatak and R.L. Malmberg, 1998. Arginine decarboxylase (polyamine synthesis) mutants of *Arabidopsis thaliana* exhibit altered root growth. Plant. J. 13: 231-239.

10 Waterman PM. 1998. Chemical taxonomy of alkaloids. In: Roberts MF, Wink M, eds., Alkaloids: Biochemistry, Ecology, and Medicinal Applications. New York: Plenum Press, 87-107.

15 Cizewski-Culotta, V. and Hamer, D.H. 1989. Fine mapping of a mouse metallothionein gene metal response element. Mol. Cell. Biol. 9: 1376-1380.

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA miniprep: version II. Plant Mol. Biol. Rep. 1: 19-21.

20 Foster, R., Izawa, T. and Chua, N.H. 1994. Plant bZIP proteins gather at ACGT elements. FASEB J. 8: 192-200.

Gerstel, D.U. 1960. Segregation in new allopolyploids of *Nicotiana*. I. Comparison of 6 x (*N. tabacum* x *tomentosiformis*) and 6 x (*N. tabacum* x *otophora*). Genetics 45: 1723-1734.

25

Gerstel, D.U. 1963. Segregation in new allopolyploids of *Nicotiana*. II. Discordant ratios from individual loci in 6 x (*N. tabacum* x *N. sylvestris*). Genetics 48: 677-689.

30 Hashimoto, T., Tamaki, K., Suzuki, K. and Yamada, Y. 1998b. Molecular cloning of plant spermidine synthases. Plant Cell Physiol. 39: 73-79.

Hibi, N., Fujita, T., Hatano, M., Hashimoto, T. and Yamada, Y. 1992. Putrescine - methyltransferase in cultured roots of *Hyoscyamus albus*. *n*-Butylamine as a potent inhibitor of the transferase both *in vitro* and *in vivo*. Plant Physiol. 100: 826-835.

- Kenton, A., Parokonny, A.S., Gleba, Y.Y. and Bennett, M.D. 1993. Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. *Mol. Gen. Genet.* 240: 159-169.
- Kim, S.-R., Choi, J.-L., Costa, M.A. and An, G. 1992. Identification of G-box sequence as an essential element for methyl jasmonate response of potato proteinase inhibitor II promoter. *Plant Physiol.* 99: 627-631.
- Kinoshita, T., Imamura, J., Nagai, H. and Shimotohno, K. 1992. Quantification of gene expression over a wide range by the polymerase chain reaction. *Anal. Biochem.* 206: 231-235.
- Lalanne, E., Mathieu, C., Vedel, F. and De Paepe, R. 1998. Tissue-specific expression of genes encoding isoforms of the mitochondrial ATPase β subunit in *Nicotiana sylvestris*. *Plant Mol. Biol.* 38: 885-888.
- Legg, P.D. and Collins, G.B. 1971. Inheritance of percent total alkaloids in *Nicotiana tabacum* L. II. Genetic effect of two loci in Burley 21 x LA Burley 21 populations. *Can. J. Genet. Cytol.* 13: 287-291.
- Leitch, I.J. and Bennett, M.D. 1997. Polyploidy in angiosperms. *Trends Plant Sci.* 2: 470-476.
- Li, W.-H. and Graur, D. 1991. *Fundamentals of Molecular Evolution*. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Mason, H.S., DeWald, D.B. and Mullet, J.E. 1993. Identification of a methyl jasmonate-responsive domain in the soybean *vspB* promoter. *Plant Cell* 5: 241-251.
- Menkens, A.E., Schindler, U. and Cashmore, A.R. 1995. The G-box: a ubiquitous regulatory element in plants bound by the GBF family of bZip proteins. *Trends Biochem. Sci.* 20: 506-510.
- Nakajima, K., Hashimoto, T. and Yamada, Y. 1993. Two tropinone reductases with different stereospecificities are short-chain dehydrogenases evolved from a common ancestor. *Proc. Natl. Acad. Sci. USA* 90: 9591-9595.
- Okamura, J.K. and Goldberg, R.B. 1985. Tobacco single-copy DNA is highly homologous to

sequences present in the genomes of its diploid progenitors. *Mol. Gen. Genet.* 198: 290-298.

Ramsey, J. and Schemske, D.W. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Syst.* 29: 467-501.

5

Rouster, J., Leah, R., Mundy, J. and Cameron-Mills, V. 1997. Identification of a methyl jasmonate-responsive region in the promoter of a lipoxygenase 1 gene expressed in barley grain. *Plant J.* 11: 513-523.

10

Saunders, J.W. and Bush, L.P. 1979. Nicotine biosynthetic enzyme activities in *Nicotiana tabacum* L. genotypes with different alkaloid levels. *Plant Physiol.* 64: 236-240.

15

Shinshi, H., Wenzler, H., Neuhaus, J.-M., Felix, G., Hofsteenge, J. and Meins, F. 1988. Evidence for N- and C-terminal processing of a plant defense-related enzyme: Primary structure of tobacco prepro- β -1,3-glucanase. *Proc. Natl. Acad. Sci. USA* 85: 5541-5545.

20

Sperisen, C., Ryals, J. and Meins, F. 1991. Comparison of cloned genes provides evidence for intergenomic exchange of DNA in the evolution of a tobacco glucan endo-1,3- β -glucosidase gene family. *Proc. Natl. Acad. Sci. USA* 88: 1820-1824.

25

Staiger, D., Kaulen, H. and Schell, J. 1989. A CACGTG motif of the *Antirrhinum majus* chalcone synthase promoter is recognized by an evolutionary conserved nuclear protein. *Proc. Natl. Acad. Sci. USA* 86: 6930-6934.

30

Suzuki, K., Yun, D.-J., Chen, X.-Y., Yamada, Y. and Hashimoto, T. 1999b. An *Atropa belladonna* hyoscyamine 6 β -hydroxylase gene is differentially expressed in the root pericycle and anthers. *Plant Mol. Biol.* 40: 141-152.

Thiele, D.J. 1992. Metal-regulated transcription in eukaryotes. *Nucl. Acids Res.* 20: 1183-1191.

Thompson, J.D. and Lumaret, R. 1992. The evolutionary dynamics of polyploid plants: origins, establishment and persistence. *Trends Ecol. Evol.* 7: 302-307.

Vaucheret, H., Vincentz, M., Kronenberger, J., Caboche, M. and Rouze, P. 1989. Molecular

cloning and characterisation of the two homeologous genes coding for nitrate reductase in tobacco.
Mol. Gen. Genet. 216: 10-15.

- Williams, M.E., Foster, R. and Chua, N.H. 1992. Sequences flanking the hexameric G-box core
5 CACGTG affect the specificity of protein binding. Plant Cell 4: 485-496.

What is claimed is:

1. An isolated DNA molecule comprising the nucleotide sequence of (SEQ. ID. NO. 2), (SEQ. ID. NO. 5), (SEQ. ID. NO. 8), (SEQ. ID. NO. 11), (SEQ. ID. NO. 13), (SEQ. ID. NO. 15), (SEQ. ID. NO. 18), (SEQ. ID. NO. 21), (SEQ. ID. NO. 23), (SEQ. ID. NO. 25) or (SEQ. ID. NO. 26) or
5 comprising a nucleotide sequence encoding the amino acid sequence encoded by (SEQ ID NO. 3), (SEQ. ID. NO. 6), (SEQ ID. NO. 9), (SEQ. ID. NO. 12), (SEQ. ID. NO. 14), (SEQ. ID. NO. 16), (SEQ. ID. NO. 19), (SEQ. ID. NO. 22) OR (SEQ. ID. NO. 24).
- 10 2. A vector comprising the isolated DNA molecule of claim 1 operably linked to sequences capable of directing the transcription of a mRNA encoded by said isolated DNA molecule.
3. An isolated DNA molecule comprising a DNA sequence complementary to the nucleotide sequence of claim 1.
- 15 4. A vector comprising the isolated DNA molecule of claim 3 operably linked to sequences capable of directing the transcription of a mRNA encoded by said isolated DNA molecule.
5. A cultured transgenic tobacco cell stably transformed with the vector of claim 2.
- 20 6. A cultured transgenic tobacco cell stably transformed with the vector of claim 4.
7. A transgenic tobacco plant stably transformed with the vector of claim 2.
- 25 8. A transgenic tobacco plant stably transformed with the vector of claim 4.
9. The isolated DNA molecule of claim 1, wherein the isolated DNA molecule comprises the nucleotide sequence of (SEQ ID NO:).
- 30 10. A vector comprising the isolated DNA molecule of claim 9 operably linked to sequences capable of directing the transcription of a mRNA encoded by said isolated DNA molecule.
11. An isolated DNA molecule comprising a DNA sequence complementary to the nucleotide sequence of the isolated DNA molecule of claim 9.

12. An isolated DNA sequence comprising about a fifteen to about a twenty-five base pair oligonucleotide sequence identical to any consecutive about fifteen to about twenty-five base pair sequence found in (SEQ. ID. NO. 2), (SEQ. ID. NO. 5), (SEQ. ID. NO. 8), (SEQ. ID. NO. 11), (SEQ. ID. NO. 13), (SEQ. ID. NO. 15), (SEQ. ID. NO. 18), (SEQ. ID. NO. 21), (SEQ. ID. NO. 23),
5 (SEQ. ID. NO. 25) or (SEQ. ID. NO. 26).

13. A cultured transgenic tobacco cell stably transformed with the vector of claim 10.

14. A transgenic tobacco plant stably transformed with the vector of claim 10.

15. A vector comprising a DNA sequence which encodes an antisense mRNA which is complementary to a fragment of a mRNA encoded by the isolated DNA molecule of claim 1, wherein said sequence is operably linked to sequences capable of directing the transcription of said antisense mRNA in tobacco cells and wherein the expression of said antisense mRNA in tobacco
15 cells is sufficient to provide for reduced nicotine content in tobacco cells which are stably transformed with said vector as compared to untransformed tobacco cells.

16. A cultured transgenic tobacco cell stably transformed with the vector of claim 15.

17. An isolated and purified protein comprising the amino acid sequence identified in (SEQ ID NO. 3), (SEQ. ID. NO. 6), (SEQ ID. NO. 9), (SEQ. ID. NO. 12), (SEQ. ID. NO. 14), (SEQ. ID. NO. 16), (SEQ. ID. NO. 19), (SEQ. ID. NO. 22) or (SEQ. ID. NO. 24).

18. A method for regulating gene expression in a plant comprising functionally linking an alkaloid gene promoter to a nucleic acid encoding a protein, wherein the promoter comprises a nucleic acid sequence selected from the group consisting of the sequences identified in (SEQ ID NO. 1), (SEQ. ID. NO. 4), (SEQ ID. NO. 7), (SEQ. ID. NO. 10), (SEQ. ID. NO. 17), and (SEQ. ID. NO. 20).

19. The method of claim 18, wherein the nucleic acid encoding a protein encodes a protein involved in the biosynthesis of alkaloids in plants.

20. A plant transformed by the method of claim 18.

1 / 21

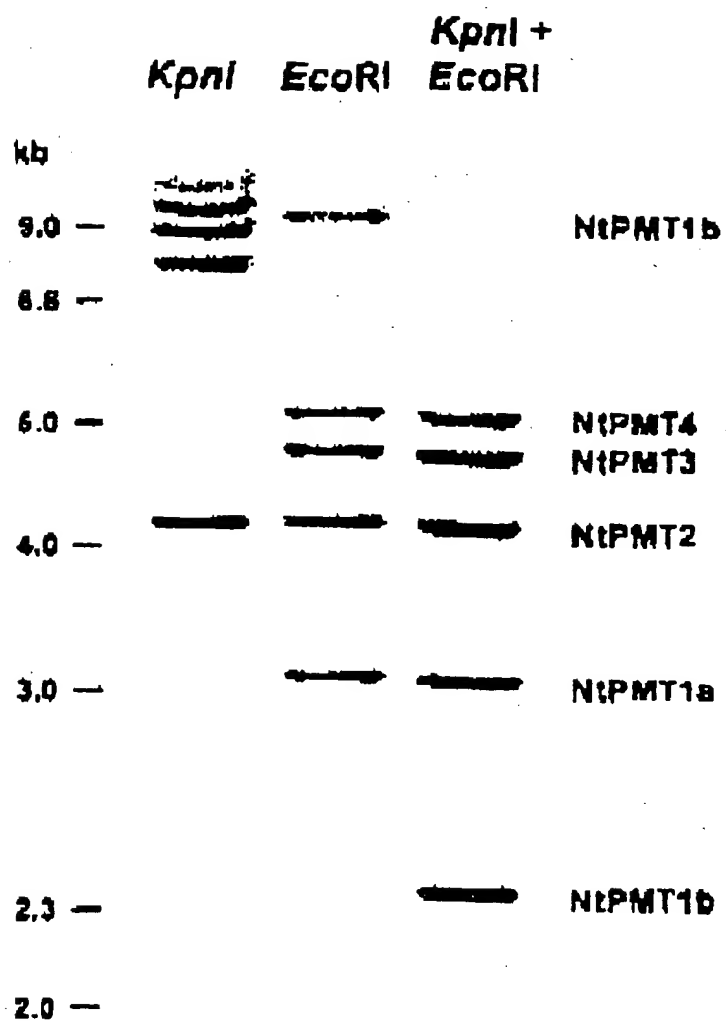


Figure 1

Exon 1

NIPMT4	MEVI	STNT	NGST	FKNGAI	PMNGHQ	NGTS	HLNGYQNGTSKHO	...
NIPMT3	MEVI	STNT	NGST	FKNGAI	PM		NGYQNGTSKHO	...
NIPMT1a	MEVI	STNT	NGST	FKNGAI	PMNGHQ	NGTS	HLNGYQNGTSKHO	...
NIA411	MEVI	STNT	NGST	FKNGAI	PMNGHQ	NGTS	HLNGYQNGTSKHO	...
NIPMT2	MEVI	STNT	NGST	FKNGAI	PMNGHQ	NGTS	HLNGYQNGTSKHO	...

[illegible]

NtPMT4	Q	N	G	N	G	T	S	H	D	N	G	N	E	.	.	L	L	G	S	S	N	S	I	K	G	W	F	S	E	F	S	A	L	W	P	G	
NtPMT3	Q	N	G	N	G	T	S	H	D	N	G	N	E	.	.	L	L	G	S	S	N	S	I	K	P	G	W	F	S	E	F	S	A	L	W	P	G
NtPMT1a	Q	N	G	N	G	T	S	H	D	N	G	N	E	.	.	L	L	G	S	S	N	S	I	K	P	G	W	F	S	E	F	S	A	L	W	P	G
NtA411	Q	N	G	N	G	T	S	H	D	N	G	N	E	.	.	L	L	G	S	S	N	S	I	K	P	G	W	F	S	E	F	S	A	L	W	P	G
NtPMT2	Q	N	G	N	G	T	S	H	D	N	G	N	E	.	.	L	L	G	S	S	N	S	I	K	P	G	W	F	S	E	F	S	A	L	W	P	G

Exon 2

Exon 3

NtPMT4	EAFSLKVEKLLLFQGGKSDYQDVMLFESATYGKVLTLDGAIQHTENGGFP
NtPMT3	EAFSLKVEKLLLFQGGKSDYQDVMLFESATYGKVLTLDGAIQHTENGGFP
NtPMT1a	EAFSLKVEKLLLFQGGKSDYQDVMLFESATYGKVLTLDGAIQHTENGGFP
NtA411	EAFSLKVEKLLLFQGGKSDYQDVMLFESATYGKVLTLDGAIQHTENGGFP
NtPMT2	EAFSLKVEKLLLFQGGKSDYQDVMLFESATYGKVLTLDGAIQHTENGGFP

NtPMT4	Y	T	E	M	I	V	H	L	P	L	G	S	I	P	N	P	K	K	V	L	I	I	G	G	G	G	F	T	L	F	E	M	L	R	Y	P	T	I	E	K	I	D	I	V	E	I	D
NtPMT3	Y	T	E	M	I	V	H	L	P	L	G	S	I	P	N	P	K	K	V	L	I	I	G	G	G	G	F	T	L	F	E	M	L	R	Y	P	T	I	E	K	I	D	I	V	E	I	D
NtPMT1a	Y	T	E	M	I	V	H	L	P	L	G	S	I	P	N	P	K	K	V	L	I	I	G	G	G	G	F	T	L	F	E	M	L	R	Y	P	T	I	E	K	I	D	I	V	E	I	D
NtA411	Y	T	E	M	I	V	H	L	P	L	G	S	I	P	N	P	K	K	V	L	I	I	G	G	G	G	F	T	L	F	E	M	L	R	Y	P	T	I	E	K	I	D	I	V	E	I	D
NtPMT2	Y	T	E	M	I	V	H	L	P	L	G	S	I	P	N	P	K	K	V	L	I	I	G	G	G	G	F	T	L	F	E	M	L	R	Y	P	T	I	E	K	I	D	I	V	E	I	D

Exon 4

Exon 5

NIPMT4	DVVVDVSRK	KFFPYLAANFNDPRVTLVL	GDGAAFVKAAQAAGYYDAI	I	V	D
NIPMT3	DVVVDVSRK	KFFPYLAANFNDPRVTLVL	GDGAAFVKAAQAAGYYDAI	I	V	D
NIPMT1a	DVVVDVSRK	KFFPYLAANFNDPRVTLVL	GDGAAFVKAAQAAGYYDAI	I	V	D
NIA411	DVVVDVSRK	KFFPYLAANFNDPRVTLVL	GDGAAFVKAAQAAGYYDAI	I	V	D
NIPMT2	DVVVDVSRK	KFFPYLAANFNDPRVTLVL	GDGAAFVKAAQAAGYYDAI	I	V	D

Exon 6

	SSDP	GPAKDLFERPFF	FE	AVAKALRP	GGVVCT	QAES	WL	HMH	I	KQ	I
NtPMT4	SSDP	GPAKDLFERPFF	FE	AVAKALRP	GGVVCT	QAES	WL	HMH	I	KQ	I
NtPMT3	SSDP	GPAKDLFERPFF	FE	AVAKALRP	GGVVCT	QAES	WL	HMH	I	KQ	I
NtPMT1a	SSDP	GPAKDLFERPFF	FE	AVAKALRP	GGVVCT	QAES	WL	HMH	I	KQ	I
NA411	SSDP	GPAKDLFERPFF	FE	AVAKALRP	GGVVCT	QAES	WL	HMH	I	KQ	I
NtPMT2	SSDP	GPAKDLFERPFF	FE	AVAKALRP	GGVVCT	QAES	WL	HMH	I	KQ	I

Exon 7

NtPMT4	A	N	C	R	Q	V	F	K	G	S	V	N	Y	A	W	T	T	V	P	T	Y	P	T	G	V	I	G	Y	M	L	C	S	T	E	G	P	E	V	D	F	K	N	P	V	N	P	I	D
NtPMT3	A	N	C	R	Q	V	F	K	G	S	V	N	Y	A	W	T	T	V	P	T	Y	P	T	G	V	I	G	Y	M	L	C	S	T	E	G	P	E	V	D	F	K	N	P	V	N	P	I	D
NtPMT1a	A	N	C	R	Q	V	F	K	G	S	V	N	Y	A	W	T	T	V	P	T	Y	P	T	G	V	I	G	Y	M	L	C	S	T	E	G	P	E	V	D	F	K	N	P	V	N	P	I	D
NtA411	A	N	C	R	Q	V	F	K	G	S	V	N	Y	A	W	T	T	V	P	T	Y	P	T	G	V	I	G	Y	M	L	C	S	T	E	G	P	E	V	D	F	K	N	P	V	N	P	I	D
NtPMT2	A	N	C	R	Q	V	F	K	G	S	V	N	Y	A	W	T	T	V	P	T	Y	P	T	G	V	I	G	Y	M	L	C	S	T	E	G	P	E	V	D	F	K	N	P	V	N	P	I	D

Ехол 8

NIPMT4	K	E	T	T	Q	V	K	S	K	L	A	P	L	K	F	Y	N	S	D	I	H	K	A	A	F	I	L	P	S	F	A	R	S	M	I	E	S
NIPMT3	K	E	T	T	Q	V	K	S	K	L	A	P	L	K	F	Y	N	S	D	I	H	K	A	A	F	I	L	P	S	F	A	R	S	M	I	E	S
NIPMT1a	K	E	T	T	Q	V	K	S	K	L	A	P	L	K	F	Y	N	S	D	I	H	K	A	A	F	I	L	P	S	F	A	R	S	M	I	E	S
NA411	K	E	T	T	Q	V	K	S	K	L	A	P	L	K	F	Y	N	S	D	I	H	K	A	A	F	I	L	P	S	F	A	R	S	M	I	E	S
NIPMT2	K	E	T	T	Q	V	K	S	K	L	A	P	L	K	F	Y	N	S	D	I	H	K	A	A	F	I	L	P	S	F	A	R	S	M	I	E	S

Figure 2

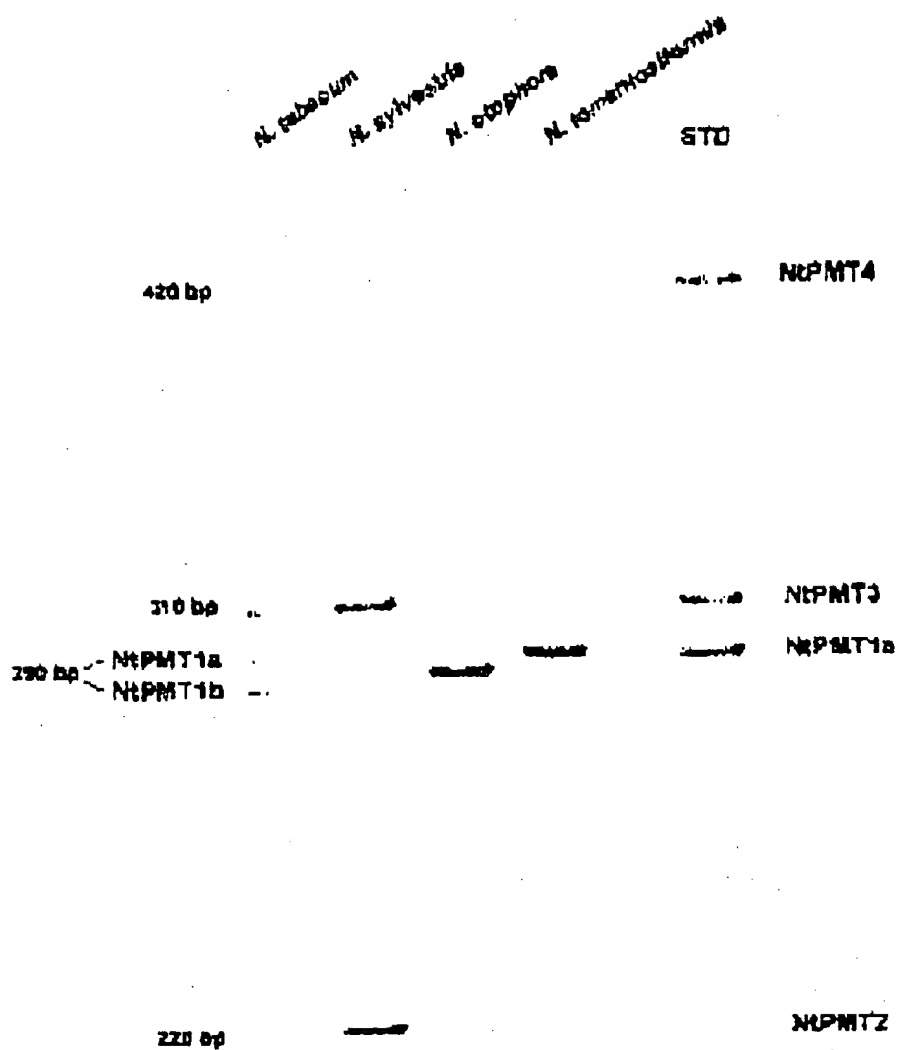


Figure 3

4 / 21

NtPMT3	-----	-----	-----gc	tgtacaaaag	gatgtctcaa	atcatttgga	atattaattc	-1029	
NtPMT2	-----	-----	-----	-----	-----	-----	-----ctgaggtg	-1039	
NtPMT4	-----	-----	-----	-----	-----	-----	-----		
NtPMT1a	-----	-----	-----	-----	-----	-----	-----		
G-box									
NtPMT3	tgcaatcaac	aagaaatacc	ccactattaa	gaccattat	cactggcaca	aaaattatga	gatcattaaa	catctttaa	-949
NtPMT2	acaagaacaa	tctcgtgtga	atcagatgga	tgagataat	agaggtgggt	ggaatctata	accaaagcag	ctgggttgat	-959
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	-----	-----	-----	-----	-----	-----	-----	-----	
G-box									
NtPMT3	ctgtccctat	ttggaagagt	gtggttatggg	agatgcctcc	caggagtacc	taaagctgaa	tatgatggaa	gttttaacaa	-869
NtPMT2	gactgtgcga	gttgacagaa	caattgaagg	gtcattttgt	gaatttgggg	ccatttcaaa	ggaaaaagaa	aagatgactt	-879
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	-----	-----	-----	-----	-----	-----	-----	-----	
G-box									
NtPMT3	acaaattggg	aagcagggat	tgagggatcc	tcagagatga	agagggaggc	tttgtcatgg	cttttccgat	gcctataatc	-789
NtPMT2	agcatttaata	aatcaaatga	aaataaggct	tagcgttaaa	atcaaaaggaa	atggcaagcc	tggtcctctg	agcaatgctt	-799
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	-----	-----	-----	-----	-----	-----	-----	-----	
G-box									
NtPMT3	tataataaca	tcagtgaagc	agaattgaaa	gccatcaagt	atgggtgtga	atgggtgcaa	tacaaaggaa	tatcaaaact	-709
NtPMT2	ctgaggacag	tagtaaaaac	aatatcagac	aaaaagtaaa	gttgtattat	ttagcttgag	gataaagtat	gtcattagtt	-719
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	-----	-----	-----	-----	-----	-----	-----	-----	
G-box									
NtPMT3	cattgtggaa	actgactcga	ggatgatcta	tgacatacta	cagaccaaaa	ctcaagcaa	caacaagtgt	aaacaagaga	-629
NtPMT2	ttgtgagaga	tttgggtgcc	tctacaatga	ttgttgaagt	ccctatttat	ctcctacac	aggaaacaaa	atcctaggat	-639
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	-----	-----	-----	-----	-----g	cttcaatgg	agaaggaaaa	tatttccagt	-625
G-Box									
NtPMT3	ccgagaaatt	aatggagatt	ctggacccct	gcaggacacc	tgttaccat	tgccttcgcg	aagcaatca	agggcgagac	-549
NtPMT2	caaagccctc	ttaaatgaca	ataatgggtt	taatgatgaa	tatgtagcgg	catgacatga	atgcccaat	tcacgcaac	-559
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	gtaaacacaa	gtgaatgaag	agaagccaaa	ataatctcta	tcattcaagc	cttagtgga	gattaaaa	atgatttact	-545
PAL									
NtPMT3	tggtttgcta	aagggccac	cagagctaac	gaaggtatcc	ctcatcaga	ttttagacag	gtatcaaaag	cgcccaaggg	-469
NtPMT2	gactatttat	ttatatttga	ggaatatttt	ttattatgaa	ctatctgggt	acaagcattc	gtttgtctcc	gttgattacg	-479
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	ttcttatcaa	agtataggt	gatcaacagc	tttcgtgaaa	gtcattagg	agaatattat	aatctctttt	atgctgaaga	-465
G-box									
NtPMT3	ccctttcttc	atggatatgc	ggcaggctcc	ttattttaga	attagatgtg	aaaaactaa	tttttttttg	taagtttaatt	-389
NtPMT2	ttgattttgg	gatctacttc	ataccaacgg	aagccgttgt	ccttgacttt	cgtttcatt	taattcatct	tcctgtgtcc	-399
NtPMT4	-----	-----	-----	-----	-----	-----	-----	-----	
NtPMT1a	acccacacaa	ggaagatcaa	aaaatacatg	actttcagat	gacttcctgg	agctttattt	ttaaagagtgt	gctaggtggt	-385
G-box									
NtPMT3	ctgtgatag	tgagaggaaa	tcgtctaat	tgatattttt	cccttagacc	cttctctccc	ttaggttaaa	aggtagctcc	-309
NtPMT2	tccgttttca	caagtgaagc	accattctca	ttatttgaag	gaacccaatt	cttacctata	caaatgttac	atcattcgtt	-319
NtPMT4	ctctgttcca	caagtgaagc	accattctca	ttatttgaag	gaacccaatt	cttacctata	caaatgttac	atcattcgtt	-319
NtPMT1a	cagcaagag	gtcttgga	gatattcata	aattttgaa	ttatttggta	taagaggagag	atgggcacac	catgcttggt	-305
G-box									
NtPMT3	gaggttaaggt	ttatgttccc	ctggtgttaa	tttttttttg	tttatatata	gacatggtat	gggtccagct	aaacccccc	-229
NtPMT2	aaatcttcta	cttggattata	aagagttttg	ttcggaggagt	aaacagatgc	gaagaaagaa	agcagagatc	taagaaattc	-239
NtPMT4	aaatcttcta	cttggattata	aagagttttg	ttcggaggagt	aaacagatgc	gaagaaagaa	agcagagatc	taagaaattc	-239
NtPMT1a	aaagagtaa	gaggaagaaa	ggagacagaa	gaggaatag	tttggggggg	gggggggggg	gtttcctaaa	caagaaattc	-225
G-box									
NtPMT3	caccacaggg	gaggaatacc	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	-153
NtPMT2	ttcaaaagag	gaggaagaaa	tgaaatcaca	catgtactaa	aaatcttagg	gtactacttt	actataatct	ggcagagac	-159
NtPMT4	ttcaaaagag	gaggaagaaa	tgaaatcaca	catgtactaa	aaatcttagg	gtactacttt	actataatct	ggcagagac	-159
NtPMT1a	ttcaaaagag	gaggaagaaa	tgaaatcaca	catgtactaa	aaatcttagg	gtactacttt	actataatct	ggcagagac	-156
CCAAT-Box									
NtPMT3	aaatcttcta	cttggattata	aagagttttg	ttcggaggagt	aaacagatgc	gaagaaagaa	agcagagatc	taagaaattc	-84
NtPMT2	aaatcttcta	cttggattata	aagagttttg	ttcggaggagt	aaacagatgc	gaagaaagaa	agcagagatc	taagaaattc	-82
NtPMT4	aaatcttcta	cttggattata	aagagttttg	ttcggaggagt	aaacagatgc	gaagaaagaa	agcagagatc	taagaaattc	-82
NtPMT1a	aaatcttcta	cttggattata	aagagttttg	ttcggaggagt	aaacagatgc	gaagaaagaa	agcagagatc	taagaaattc	-84
MRE									
NtPMT3	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
NtPMT2	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
NtPMT4	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
NtPMT1a	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
TATA-Box									
NtPMT3	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
NtPMT2	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
NtPMT4	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
NtPMT1a	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	-4
+1									
NtPMT3	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	+60
NtPMT2	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	+77
NtPMT4	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	+77
NtPMT1a	tggtgatgtg	gtttattttt	aaaaaa--	aaatctta	actataatct	gcctggagac	aaacccccc	aaacccccc	+60

Figure 4

5 / 21

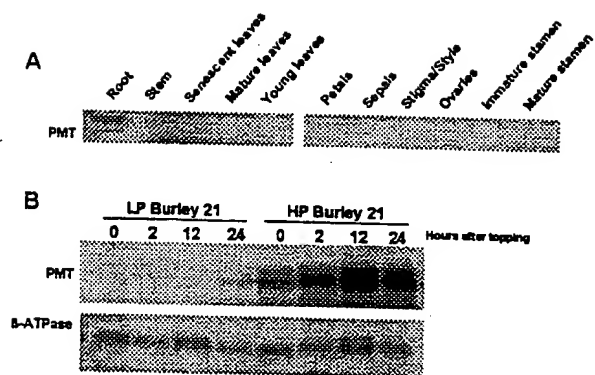


Figure 5

6 / 21

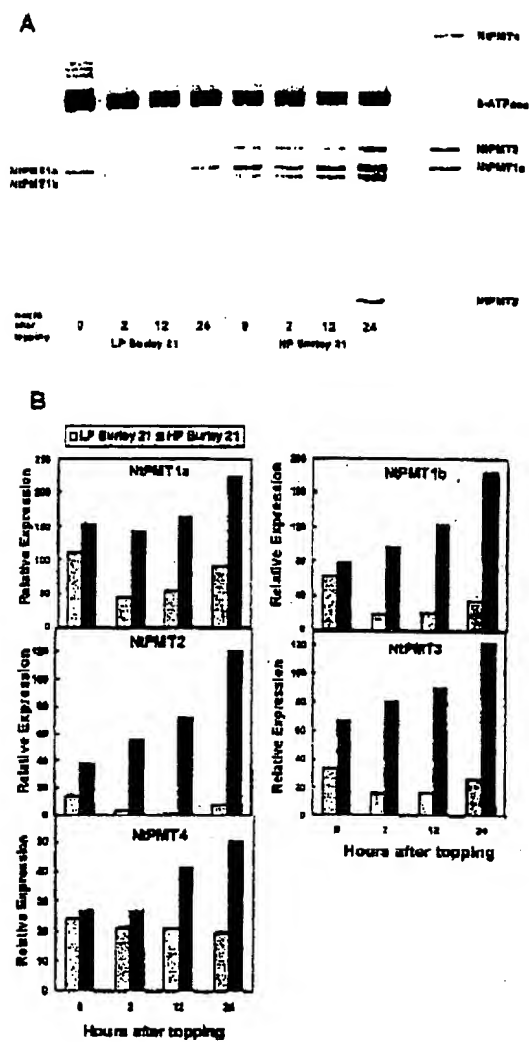


Figure 6

```

NtADC1
ttcacgttctcttctcaattcccataaaaagaacccttcggttag' 319
gtttccgctcctatttt--ctcttcttctacgcttc 78
NtADC2
.....c.....
.....tc...a.....c
..t... 80

NtADC1
ctcttctgatatacaatatctgtatgggtgttttcttg
ttcgaattttagatttgtttgcctttaataacctgta
acctta 158
NtADC2
.....a.....
.....t.....a..
..... 160

NtADC1
taattctctgttttaaaccaaaaacttagcttcttctg
aagtcaggggtggggatatttggatcgtgtaagagtgt
gttaga 238
NtADC2 -
.....
.....t.....
..... 239

NtADC1
agggtgattatcttttgattcagttcctttttgcttc
ttttgagggggttagccggggcctcggcctcggcgggt
tttaat 318
NtADC2
G.....

NtADC1
agcccccatctattacaaccattgggcaaaaacatca
ttaaatctgtacaaagcaaaccttaatttagtttaa
ttttct 398
NtADC2
.....t.....
.....a.....
..... 399

1
M P A L G C C V D A T -
V S P P
NtADC1
gtattcttttgattcttttaacagaagaagaagagATGC
CGGCCCTAGGTTGTTGCGTAGACGCTACT---
GTTTCCCCCTCC 475
NtADC2
.....a.....t.....ATG.
.....T.....T...G..GTT.....
..... 479

1
M * * * * * * * * A V
* * * *

16 L G Y A F S R D S S
L P A P E F F T S G V P P
T N S A
NtADC1
CTCGGCTATGCCTTCTCTCGGGATAGCTCTCTTCCCCG

```

Figure 7 (a)

8 / 21

CGCCGGAGTTCTTTACCTCCGGCGTACCTCCTACAAA
CTCCG 555
NtADC2
...A.....
.....G.....G.....
...T. 559
17 * S * * * * * * * * *
* * * * * * * A * * * * *
* * * *

43 A G S I G S P D L S
S A L Y G V D G W G A P Y
F S V
NtADC1
CCGCCGGTTCCATTGGGTCTCCGGATCTGTCCTCTGC
TTTGACGGGGTCGATGGGTGGGAGCTCCTTATTTT
TCCGTT 635
NtADC2
...T.C.....T....G.....
...A.....
..T... 639
44 * A * * * * * * * * *
* * * * * * * * * * * * *
* * *

69 N S N G D I S V R P
H G T D T L P H Q E I D L
L K V V
NtADC1
AACTCTAACGGAGATATCTCCGTCCGACCACATGGTA
CGGACACACTCCCCACCAGGAAATTGACCTTCTCAA
GGTCGT 715

NtADC2
.....T.....C.....
.....T.....T.....
..... 719
70 * * * * * * * * * *
* * * * * * * * * * * *
* * * *

96 K K A S D P K N S G
G L G L Q L P L V V R F P
D V L K
NtADC1
GAAAAAGGCCTCCGACCCGAAAAATTCAGGGGGGCTC
GGGCTTCAGCTGCCTCTTGTGTTCGCTTCCCTGATG
TGCTAA 795
NtADC2
.....T.....T
.....
..T.G. 799
97 * * * * * * * * * *
* * * * * * * * * * * *
* * * *

123 N R L E S L Q S A F
D L A V H S Q G Y G A H Y
Q G V
NtADC1
AAAACCGGTTGGAATCTCTGCAATCGGCTTTTGATCT
CGCTGTTCAATCCCAGGGCTATGGGGCCCACTACCAA
GGTGTT 875
NtADC2
.....

Figure 7 (b)

...G.....
 879
 124 * * * * *
 * * * * *
 * * *
 149 Y P V K C N Q D R F
 V V E D I V K F G S S F R
 F G L E
 NtADC1
 TATCCCGTGAAATGCAATCAAGACAGGTTTCGTGGTGG
 AAGATATTGTCAAATTCGGGTCGTCATTCCGGTTCGG
 GTTGGA 955
 NtADC2

C.....C.....
 959
 150 * * * * *
 * * * * * P * *
 * * * *
 176 A G S K P E L L L A
 M S C L C R G S A E G L L
 V C N G
 NtADC1
 AGCTGGGTCTAAACCCGAGCTCCTGTAGCCATGAGC
 TGTCTCTGCAGGGGCAGTGCTGAGGGCCTTCTCGTTT
 GCAATG 1035
 NtADC2
 ...C.....
A.....
 1039

177 * * * * *
 * * * * * K * * * * *
 * * * *
 203 F K D A E Y I S L A
 L V A R K L M L N T V I V
 L E Q
 NtADC1
 GTTTCAGGACGCTGAGTACATTTCGCTTGCTTTGGT
 TGCAAGAAAGCTCATGTTAAACACTGTAATTGTTCTT
 GAACAA 1115
 NtADC2

G...
 1119
 204 * * * * *
 * * * * *
 * * *
 229 E E E L D L V I D I
 S R K M A V R P V I G L R
 A K L R
 NtADC1
 GAGGAGGAGCTTGACCTTGTGATTGATATAAGCCGTA
 AGATGGCTGTTTCGGCCCGTAATTGGACTTCGGGCTAA
 GCTCAG 1195
 NtADC2
A..
T.....
 1199
 230 * * * * *
 * H * * * * *
 * * * *

```

256 T K H S G H F G S T
S G E K G K F G L T T T Q
I V R V
NtADC1
GACCAAGCATTGAGGCCATTTGGATCCACTTCTGGA
GAAAAAGGTAAGTTTGGGCTTACAACGACCCAAATTG
TTCGTG 1275
NtADC2
.....
..... 1279
257 * * * * *
* * * * *
* * * * *

283 V K K L E E S G M L
D C L Q L L H F H I G S Q
I P S
NtADC1
TAGTGAAGAAGCTGGAAGAATCCGGAATGCTGGATTG
CCTTCAGTTGCTGCATTTTCACATTGGATCTCAGATC
CCTTCA 1355
NtADC2
.G.....A.....
T.....
.....T 1359
284 * * * * *
* * * * *
* * * * *

309 T A L L A D G V G E
A A Q I Y C E L I R L G A
G M K F
NtADC1
ACGGCGTTGCTTGCTGATGGTGTGGTGAGGCTGCTC
AGATTTATTGTGAATTAATCCGTCTTGGTGCGGGTAT
GAAGTT 1435
NtADC2
.....G.....A.....A.....C....
.....G.....A.....
..... 1439
310 * G * * * * *
* * * * * V * * * *
* * * *

336 I D T G G G L G I D
Y D G T K S C D S D V S V
G Y G I
NtADC1
CATTGATACTGGAGGTGGGCTCGGAATTGATTATGAT
GGTACTAAATCATGTGATTGAGATGTCTCTGTTGGCT
ATGGCA 1515
NtADC2
.....T.....T.....
.....C.....T.....
..... 1519
337 * * I * * * * *
* * * * *
* * * *

```

Figure 7 (d)

11 / 21

363 Q E Y A S T V V Q A
V Q Y V C D R K G V K H P
V I C

NtADC1

TTCAAGAATACGCCTCCACAGTTGTCCAGGCGGTTCA
ATATGTTTGGCGACCGTAAGGGCGTGAAGCACCCAGTG
ATTGTC 1595

NtADC2

.....T.....G.....T.....
.....A.....T.....A.....
..C... 1599

364 * * * * A * * * *
* * * * * * * * * *
* * *

389 S E S G R A I V S H
H S I L I F E A V S A S S
H S C S

NtADC1

AGCGAAAGTGGCAGGGCAATTGTTTCTCATCACTCAA
TTCGATTTTCGAAGCCGTGTCTGCTTCTAGTCACTC
ATGTTT 1675

NtADC2

.....
.....
..... 1679

390 * * * * * * * * * *
* * * * * * * * * *
* * * *

416 S S H L S S G G L Q
S M A E T L N E D A L A D
Y R N L

NtADC1

TTCTTCACATCTGTCTTCTGGTGGCCTCCAATCCATG
GCGGAGACGCTCAATGAAGATGCCCTTGCTGATTACC
GCAATT 1755

NtADC2

.....
.....C.....
..... 1759

417 * * * * * * * * * *
* * * * * * * * * *
* * * *

443 S A A A V R G E Y E
T C V L Y S D Q L K Q R C
V D Q

NtADC1

TATCTGCTGCTGCAGTTCGTGGAGAGTACGAGACGTG
TGTACTTTTACTCTGATCAGTTGAAACAGAGATGTGTG
GATCAG 1835

NtADC2

.....T.....A..
.....
..... 1839

444 * * * * * * * * * *
* * * * * * * * * *
* * *

469 F K E G S L G I E H
L A A V D S I C D F V S K
A M G A

NtADC1

TTTAAAGAAGGGTCCTTGGGTATTGAACATCTTGCTG

Figure 7 (e)

12 / 21

CTGTTGATAGCATCTGTGATTTTGTATCAAAGGCTAT
GGGGGC 1915
NtADC2
.....
..... 1919
470 * * * * *
* * * * *
* * * * *

496 A D P I R T Y H V N
L S I F T S I P D F W A F
G Q L F
NtADC1
TGCTGATCCTATCCGCACTTACCATGTGAATCTGTCA
ATTTTCACTTCAATTCTGATTTTGGGCCTTTGGTC
AATTGT 1995
NtADC2
.....G.....
.....
..... 1999
497 * * * V * * * * *
* * * * *
* * * * *

523 P I V P I H R L D E
K P A V R G I L S D L T C
D S D
NtADC1
TTCCGATTGTTCCAATACACCGTTTAGATGAAAAGCC
TGCAGTAAGGGGAATATTATCGGACTTGACTTGTGAC
AGTGAT 2075

NtADC2
.....T.....C.....
.....G.....A.....
..... 2079
524 * * * * *
* * * * *
* * * * *

549 G K V D K F I G G E
S S L Q L H E L G S N G D
G G G Y
NtADC1
GGGAAGGTTGATAAGTTTCATTGGTGGCGAATCAAGCT
TGCAGCTGCATGAATTGGGAAGTAATGGCGATGGTGG
TGGGTA 2155
NtADC2
.....
...C...A.....
...T.. 2159
550 * * * * *
* * * P * * * * *
* * * * *

576 Y L G M F L G G A Y
E E A L G G L H N L F G G
P S V V
NtADC1
TTATCTGGGGATGTTTTTGGGTGGGGCTTATGAGGAG
GCGCTCGGAGGACTCCACAACCTGTTTGGTGGACCAA
GCGTGG 2235
NtADC2
.....

Figure 7 (f)

13 / 21

.....
 .T..C. 2239
 577 * * * * *
 * * * * *
 * * * * *

603 R V V Q S D S A H S
 F A M S R S V P G P S C A
 D V L
 NtADC1
 TGC GCGTGGTGCAGAGCGATAGCGCTCACAGCTTCGC
 CATGTCTCGCTCCGTCCCTGGCCCGTCCTGCGCGGAC
 GTGCTC 2315
 NtADC2

.....T..
A.....T.....T.T.
 2319
 604 * * * * *
 * * * T * * * * *
 * * *

629 R A M Q H E P E L M
 F E T L K H R A E E F L E
 Q E E D
 NtADC1
 CGAGCGATGCAGCACGAGCCCGAGCTCATGTTCGAGA
 CTCTCAAGCACCGTGCGGAGGAATTCTTGGAACAAGA
 AGAAGA 2395
 NtADC2

.....

 ...T.. 2399

630 * * * * *
 * * * * *
 * * D *

656 K G L A I A S L A S
 S L A Q S F H N M P Y L V
 A P A S
 NtADC1
 CAAAGGGCTGGCCATTGCATCTTTGGCCAGCAGCTTA
 GCTCAGTCCTTCCATAACATGCCTTACCTTGTGGCGC
 CTGCAT 2475
 NtADC2
TG...A.....G..

 ..T... 2479
 657 * * * * V E * * * *
 * V * * * * * * * * *
 * * S *

683 C C F T A V T A N N
 G G Y N Y Y Y S D E N A A
 D S A
 NtADC1
 CTTGCTGCTTCACTGCAGTTACTGCTAACAACGGTGG
 CTATAACTACTATTACAGTGATGAGAATGCAGCAGAT
 TCTGCT 2555
 NtADC2
C.....T.C.....A.....T.....
T.....
 2559
 684 * R * * * A * D * *
 * * * * * * * * *
 * * *

Figure 7(g)

14 / 21

```

      709 T G E D E I W S Y C
T A ***

NtADC1
ACAGGGGAGGATGAGATTTGGTCCTATTGCACTGCTT
GAagtgttgtagcgcacatccagtttagttgtcg
tcgaag 2635
NtADC2
.....T
GA.....C.....
....g. 2639
      710 * * * * *
* * * * *

```



```

      720
NtADC1
ttgtctgtttttgaataataacccttagttggtgatgt
ttttct
      2678
NtADC2
.....aataata.....
.....
      2682

```

Figure 7(h)

N. tabacum	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	48	N. tabacum	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	442
N. sylvestris	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	50	N. sylvestris	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	443
L. esculentum	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	50	L. esculentum	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	443
A. thaliana	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	50	A. thaliana	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	443
--- 41	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	50	--- 41	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	443
G. max	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	46	G. max	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	437
A. sativa	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	46	A. sativa	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	437
E. coli	KPALCCCVDAATVSPFLQYAEELSSLFAPETTTTTPPTTHANGSIC--	20	E. coli	ATVHDSHILIFAVASAE--HPCSEHDLSEOGLOSHASTLMDALADYML	408
N. tabacum	SPFLSSALTVQCHGAPTTVHKGDIIVLHGTDTLPHKIDLLKVV	93	N. tabacum	SAAVAGHTETVLTEDQLQKVCVO--QFEKSLGKHLAAYD--	497
N. sylvestris	SPFLSSALTVQCHGAPTTVHKGDIIVLHGTDTLPHKIDLLKVV	100	N. sylvestris	SAAVAGHTETVLTEDQLQKVCVO--QFEKSLGKHLAAYD--	490
L. esculentum	SPFLSSALTVQCHGAPTTVHKGDIIVLHGTDTLPHKIDLLKVV	100	L. esculentum	SAAVAGHTETVLTEDQLQKVCVO--QFEKSLGKHLAAYD--	490
A. thaliana	SPFLSSALTVQCHGAPTTVHKGDIIVLHGTDTLPHKIDLLKVV	100	A. thaliana	SAAVAGHTETVLTEDQLQKVCVO--QFEKSLGKHLAAYD--	490
G. max	SPFLSSALTVQCHGAPTTVHKGDIIVLHGTDTLPHKIDLLKVV	94	G. max	SAAVAGHTETVLTEDQLQKVCVO--QFEKSLGKHLAAYD--	490
A. sativa	SPFLSSALTVQCHGAPTTVHKGDIIVLHGTDTLPHKIDLLKVV	48	A. sativa	SAAVAGHTETVLTEDQLQKVCVO--QFEKSLGKHLAAYD--	490
E. coli	SPFLSSALTVQCHGAPTTVHKGDIIVLHGTDTLPHKIDLLKVV	76	E. coli	SAAVAGHTETVLTEDQLQKVCVO--QFEKSLGKHLAAYD--	490
N. tabacum	KKADPFGKGLGLQFPLVAVFVFLVGLLESQSAFLAVHGTQVYAT	143	N. tabacum	FVKAAGADPZAT--THVLSITZTFDQVQFQLFI	524
N. sylvestris	KKADPFGKGLGLQFPLVAVFVFLVGLLESQSAFLAVHGTQVYAT	130	N. sylvestris	FVKAAGADPZAT--THVLSITZTFDQVQFQLFI	527
L. esculentum	KKADPFGKGLGLQFPLVAVFVFLVGLLESQSAFLAVHGTQVYAT	130	L. esculentum	FVKAAGADPZAT--THVLSITZTFDQVQFQLFI	527
A. thaliana	KKADPFGKGLGLQFPLVAVFVFLVGLLESQSAFLAVHGTQVYAT	130	A. thaliana	FVKAAGADPZAT--THVLSITZTFDQVQFQLFI	527
G. max	KKADPFGKGLGLQFPLVAVFVFLVGLLESQSAFLAVHGTQVYAT	146	G. max	FVKAAGADPZAT--THVLSITZTFDQVQFQLFI	519
A. sativa	KKADPFGKGLGLQFPLVAVFVFLVGLLESQSAFLAVHGTQVYAT	97	A. sativa	FVKAAGADPZAT--THVLSITZTFDQVQFQLFI	434
E. coli	KKADPFGKGLGLQFPLVAVFVFLVGLLESQSAFLAVHGTQVYAT	120	E. coli	FVKAAGADPZAT--THVLSITZTFDQVQFQLFI	307
N. tabacum	QGVTFKCHQDFVVDIVFGSFFGLAGSEKPELLANCLCSEKSE	193	N. tabacum	VFDHLEAPFVAGIILDLTCDSDKIDYFIOGES--SLPMLGKSGD	571
N. sylvestris	QGVTFKCHQDFVVDIVFGSFFGLAGSEKPELLANCLCSEKSE	200	N. sylvestris	VFDHLEAPFVAGIILDLTCDSDKIDYFIOGES--SLPMLGKSGD	574
L. esculentum	QGVTFKCHQDFVVDIVFGSFFGLAGSEKPELLANCLCSEKSE	200	L. esculentum	VFDHLEAPFVAGIILDLTCDSDKIDYFIOGES--SLPMLGKSGD	574
A. thaliana	QGVTFKCHQDFVVDIVFGSFFGLAGSEKPELLANCLCSEKSE	200	A. thaliana	VFDHLEAPFVAGIILDLTCDSDKIDYFIOGES--SLPMLGKSGD	574
G. max	QGVTFKCHQDFVVDIVFGSFFGLAGSEKPELLANCLCSEKSE	196	G. max	VFDHLEAPFVAGIILDLTCDSDKIDYFIOGES--SLPMLGKSGD	562
A. sativa	QGVTFKCHQDFVVDIVFGSFFGLAGSEKPELLANCLCSEKSE	167	A. sativa	VFDHLEAPFVAGIILDLTCDSDKIDYFIOGES--SLPMLGKSGD	501
E. coli	QGVTFKCHQDFVVDIVFGSFFGLAGSEKPELLANCLCSEKSE	166	E. coli	VFDHLEAPFVAGIILDLTCDSDKIDYFIOGES--SLPMLGKSGD	357
N. tabacum	GLWCHGKDAFTISLAAVAKLKLNTTVLGESELDLVISIKODVA	243	N. tabacum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	616
N. sylvestris	GLWCHGKDAFTISLAAVAKLKLNTTVLGESELDLVISIKODVA	250	N. sylvestris	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	623
L. esculentum	GLWCHGKDAFTISLAAVAKLKLNTTVLGESELDLVISIKODVA	250	L. esculentum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	623
A. thaliana	GLWCHGKDAFTISLAAVAKLKLNTTVLGESELDLVISIKODVA	250	A. thaliana	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	623
G. max	GLWCHGKDAFTISLAAVAKLKLNTTVLGESELDLVISIKODVA	246	G. max	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	608
A. sativa	GLWCHGKDAFTISLAAVAKLKLNTTVLGESELDLVISIKODVA	197	A. sativa	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	544
E. coli	GLWCHGKDAFTISLAAVAKLKLNTTVLGESELDLVISIKODVA	216	E. coli	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	398
N. tabacum	PVIGLAAKLATKSGHGTSTGKSGKFLTTTQTVVVKLESSEKDLCL	293	N. tabacum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	637
N. sylvestris	PVIGLAAKLATKSGHGTSTGKSGKFLTTTQTVVVKLESSEKDLCL	300	N. sylvestris	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	649
L. esculentum	PVIGLAAKLATKSGHGTSTGKSGKFLTTTQTVVVKLESSEKDLCL	300	L. esculentum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	577
A. thaliana	PVIGLAAKLATKSGHGTSTGKSGKFLTTTQTVVVKLESSEKDLCL	300	A. thaliana	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	641
G. max	PVIGLAAKLATKSGHGTSTGKSGKFLTTTQTVVVKLESSEKDLCL	294	G. max	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	704
A. sativa	PVIGLAAKLATKSGHGTSTGKSGKFLTTTQTVVVKLESSEKDLCL	247	A. sativa	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	713
E. coli	PVIGLAAKLATKSGHGTSTGKSGKFLTTTQTVVVKLESSEKDLCL	246	E. coli	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	713
N. tabacum	QLHFKIGSGIPSTALLADGGLAAGITCEKLL--GAKGKIDTGGGLD	343	N. tabacum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	679
N. sylvestris	QLHFKIGSGIPSTALLADGGLAAGITCEKLL--GAKGKIDTGGGLD	348	N. sylvestris	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	609
L. esculentum	QLHFKIGSGIPSTALLADGGLAAGITCEKLL--GAKGKIDTGGGLD	348	L. esculentum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	640
A. thaliana	QLHFKIGSGIPSTALLADGGLAAGITCEKLL--GAKGKIDTGGGLD	348	A. thaliana	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	640
G. max	QLHFKIGSGIPSTALLADGGLAAGITCEKLL--GAKGKIDTGGGLD	344	G. max	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	721
A. sativa	QLHFKIGSGIPSTALLADGGLAAGITCEKLL--GAKGKIDTGGGLD	297	A. sativa	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	734
E. coli	QLHFKIGSGIPSTALLADGGLAAGITCEKLL--GAKGKIDTGGGLD	314	E. coli	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	733
N. tabacum	IDTGTSCSDSVFVGTGKATSTVYVAVVQVCDKGVKVCSEKSG	393	N. tabacum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	713
N. sylvestris	IDTGTSCSDSVFVGTGKATSTVYVAVVQVCDKGVKVCSEKSG	398	N. sylvestris	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	694
L. esculentum	IDTGTSCSDSVFVGTGKATSTVYVAVVQVCDKGVKVCSEKSG	398	L. esculentum	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	694
A. thaliana	IDTGTSCSDSVFVGTGKATSTVYVAVVQVCDKGVKVCSEKSG	398	A. thaliana	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	694
G. max	IDTGTSCSDSVFVGTGKATSTVYVAVVQVCDKGVKVCSEKSG	393	G. max	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	694
A. sativa	IDTGTSCSDSVFVGTGKATSTVYVAVVQVCDKGVKVCSEKSG	347	A. sativa	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	694
E. coli	IDTGTSCSDSVFVGTGKATSTVYVAVVQVCDKGVKVCSEKSG	343	E. coli	GGG-----TYLQFLOGATEALGGLQFQFVAVVQSDHASTANS	694

Figure 8

16 / 21

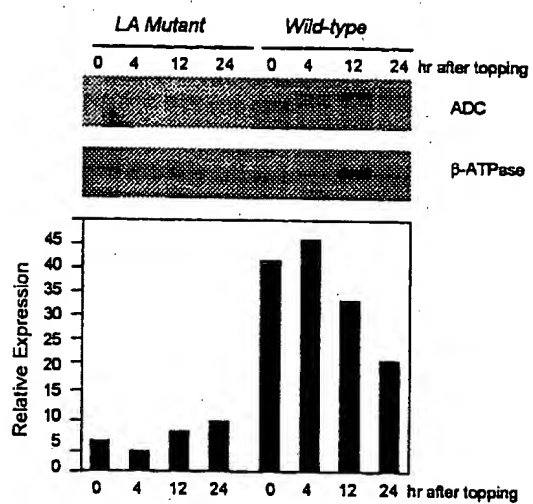


Figure 9

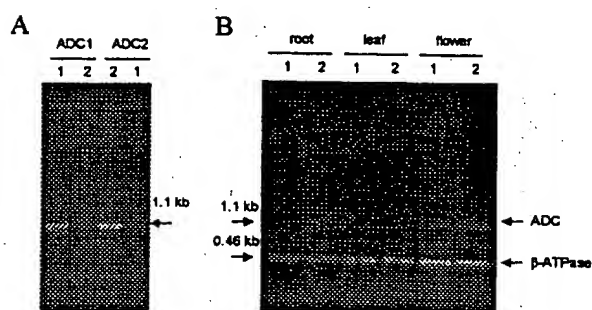


Figure 10

17 / 21

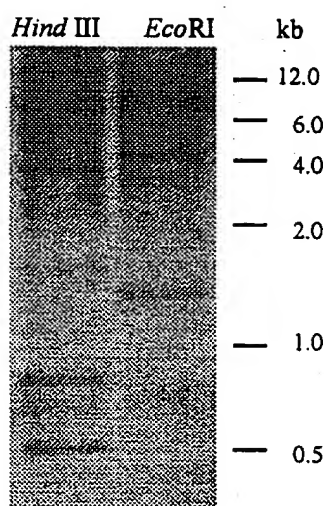


Figure 11

18 / 21

```

-135                                     -36
ODC2 CCTAGCCCTT CAACAGCTAT TTCTCTAAAA AAAAAAAAAA AAGAAGAAAA TACTACGTAG ATTACACAAT ATTATCAGTA GTAGTATCAC TTTCGTGCC
ODC1 AAGGTGAGTT TCACCAATA TGAGCGGTGT AAAAAAGCTT TTTTITTIAT TTTTITTIAT TCCTCCAAAA AACACATTTT AAGGTATTTT

-35 TATA box                               +1 * * *                                     65
ODC2 TGGGAGGAT GATAAATTT TTTAGAGTT TCCCTGTCTC AAAGGGAACA AGAAGAACAT TCATATZAT GAATCCCTAG TTCTTTTCT TTCCCTTTGA
ODC1 TAAGCACATT TTCTCTCTTT CCTTTCGGGC CTGGTATTGG ATTCTTTZAA CAATGGCTTC AACCATGTTG GCAACCTZAT CTCTTTTCT TTCCCTTTGA

66                                     165
ODC2 TTCTTCTCTC TCATTTACCT CTCTCTTTTC TTCTTTTCTT TGGATGGCGG GCCAAACAAT CATCTTTTCC GGGTTGAACC CGGGGCCCAT TCTTCAGTCC
ODC1 TTCTTCTCTC TCATTTACCT CTATCTTTTC TTACTTTTGT TGGATGGCTG GCCAAACAAT CATCTTTTCC GGGTTGAACC CAGGGGCCAT TCTTCAGTCC
pODC2 N A G Q T I I V S G L N P A A I L Q S C
pODC1 V

166                                     265
ODC2 ACAATTGGCG GCGGAGCTTC TCCTACAGCG GCGGCGCGCG CGGAAGACGG CACCAGAAAA GTCATCTCTC TGTCAAGAGA TGCCCTACAA GATTTCATGT
ODC1 ACAATTGGCG GCGGAGCTTC TCCTACAGCG GCGGCGCGCG CGGAAGACGA CACCAGAAAA GTCATCTCTC TGTCAAGAGA TGCCCTACAA GATTTCATGT
pODC2 T I G G G A S P T A A A A A E N G T R K V I P L S R D A L Q D Y K
pODC1 D

266                                     365
ODC2 TATCAATCAT AACCCAAAAA TTACAAGATG AGAACAACCC TTTTACGTTG CTAGACTTGG GTGAGGTTGT TTCTCTTATG GACCAATGGA AATTCGTCTT
ODC1 TATCAATCAT AACCCAAAAA TTACAAGATG AGAACAACCC TTTTACGTTG CTAGACTTGG GTGAGGTTGT TTCTCTTATG GACCAATGGA AATTCGTCTT
pODC2 L S I I T Q K L Q D E K Q P F Y V L D L G E V V S L M D Q W K S A L Y
pODC1 Y

366                                     465
ODC2 CCCAAATATC CTTCCATTTT ACCTGTATTA ATGTAACCTT GAACCGTGT TTCTTTCAAT TTTATCTGCT ATGGGCTCAA ATTTTGATTG TGCTAGCCGA
ODC1 CCCAAATATC CTTCCATTTT ACCTGTATTA ATGTAACCTT GAATCGTGT TTCTTTCAAT TTTATCTGCT ATGGGCTCAA ATTTTGATTG TGCTAGCCGA
pODC2 P N I R P F Y A V K C N P E P S F L S I L S A M G S N F D C A S R
pODC1 S L

466                                     565
ODC2 GCTGAAATTT AGTATGTTT ATCTCTTGGC ATTTCAGCTG ACGGTATGTT TTTCGCAAT CCATGCAATC CGGAATCCGA TATTATTTT GCAGCAAAAG
ODC1 GCTGAAATTT AGTATGTTT ATCTCTTGGC AATAAGAAAA GAGAGAGGTC AATGGGTTAC TTGATTTGAT GAAAGTTTGG GAAATTAATA TTGGGTTGT
pODC2 A E I E Y V L S L G I S P D R I V F A N P C K P E S D I I F A A K
pODC1 N K K R E R S M G Y L I .

566                                     665
ODC2 TTGGGGTGAA TCTTACATCC TATGATTCG AAGACGAGGT TTACAAGATC CGAAAGCATC ACCCGAAATC CGAATCTTGG CTCCGCATCA AGCCCATGCT
ODC1 CTTCGTATCG TCATGGGAAT CTTTAGCTGA AGTTATAACA AATTTGGAGG AGTTTCTCTT AAAAATTTGG TGCTTGGAA C AAGAACACAC
pODC2 V G V N L T T Y D S E D E V Y K I R K H R P K S E L L L R I K P H L

666                                     765
ODC2 CGACGGCAAC GCGAGATGCC CAATGGGCCG GAAATACGGC GCGCTTCAG AAGAAGTGA CCGCTGCTC CGGGCAGCTC AAGCGGCCCG TCTCACCTTA
ODC1 ATGAATAAAG CGAAGAACAC CAAGACCACT GATTTCGAAA ACACCAAAAT TCAATTTTTT TAAAGTTTTT TGCGGTGAAA TTAAGTTTTT
pODC2 D G N A R C P N G P K Y G A L P E E V D P L L R A A Q A A R L T V

766                                     865
ODC2 TCCGGCGTCT CATTCACAT CGGTAGCGGA GATGCGGATT CAACCGTTA TCTCGGCGCC ATAGCGCGCG CTAAGGAAGT GTTTGAACA GCTGCTAAAC
ODC1 CTTTTCTTTT TTAGAATGTT ATTTTATTTT TATTTATTA ATAGATTAA CATAGTTTTT TTTACTCAA ATAATATATG TCATTTTTTT ATTGCTACT
pODC2 S G V S F H I G S G D A D S H A Y L G A I A A A K E V F E T A A K

866                                     965
ODC2 TCGGGATGTC GAAATGACT GTTCTAGAGC TCGCGCGCGG GTTTACATCC GGGCACCAGT TCACACCCCG CCGCTCGCC GTTAAATCAG CTTTAAACCA
ODC1 CGCCAGCTCA CGGCGAGTGT CATTCACACA ACTTTGTAGT TTGCGCTGAT TGTAAATAA GTGCTCAAT GGAACAAAGT TCATGTAAAG TATGTGCTCA
pODC2 L G M S X N T V L D V G G G F T S G H Q F T T A A V A V K S A L K Q

966                                     1065
ODC2 ACACCTTCGAT GACGAAACCG AGTTGACAA CATAGCTGAA CCGGGTGGGT TTTTTCGAGA GACGGCGTTT ACTTTGGCAA CGAGGATTAT AGGGAAAAAG
ODC1 ATAGGAACCT TCTTAAAGTT AGGTGTCTAA ATGAAAGATG GTGCCAACTT TAAGTGTCTC CGTATGTATT CAGGCCAAAT AATGTAAAGC AAATGTAGTC
pODC2 E F D D E F E L T I I A E P G R F F A E T A F T L A T T I I G K R

1066                                     1165
ODC2 GTGAGGGGTG AATTGAGGA GTATTGGATT AACGACGGGC TGTACGGTTC GATGAACCTG GTACTTTACG ACCATGCGAC GGTGAATGCA ACGCCGTTAG
ODC1 AATAAAGCGA TGTGTCTAGA ACCAGGGGAC TCAGGGAATG CCTTACACTT TGTCCCGGCT CAACAGAAAT CTTTACTCGG AGTTTGTITT CGAAGACCAA
pODC2 V R G E L R E Y W I N D G L Y G S M N C V L Y D H A T V N A T P L

1166                                     1265
ODC2 CTGTTCTGTC GAATCTAGT AACGTTACCT GCGCGCGGCT GAAACGTTT CCGACGACTG TGTTTGGGCC CACTTGTGAT GCTCTGATA CTGTTTAAAG
ODC1 TAATTAATAGA GTGAACCTT CCTTTGAATA GGAATTCAAA AAAAAGGTGA CTTGGAACAC CAGCAAAAT TAATTCCTAG TGGGACACT GTAATAAAA
pODC2 A V L S N R S N V T C G G S K T F P T T V E G P T C D A L D T V L R

1266                                     1365
ODC2 GGATTACCAG TTACCGGAGC TGCAGGTTAA TGATTGGCTG GTTTTCTTA ATATGGGTGC TTATACTAAA GCTGCTGGGT CCAATTTTAA TGGATTTAAT
ODC1 TAATCCCTAT TTCAAAATTT TCACTTTAAT TGGAAAACT CTTCACCCA CAATCCATAA CAACACATTA TCTTTTGGAG GTGTAAAAAG GTGATGTGAC
pODC2 D Y Q L P E L Q V N D W L V F P N K G A Y T R A A G S N F N G F N

1366                                     1465
ODC2 ACTTCGCGCA TTGTTACTCA CCTCGCTTAT TCTTATCCAA GCTGATGAAC CACCTGTATT AGGAATTAAT ACCGTGTTT TGATGTTTT TTCTTTTTT
ODC1 AGCTCTAGCA ACTCTGCTGG GGGCTATTAA TAAGAAATTC AGCTTTGTAT ATTGATTTTT ATTGCGCTT TATCATGTCT TGGATATTAT TGTGTTTGGG
pODC2 T S A I V T H L A Y S Y P S .

1466                                     Poly A signal                                     1565
ODC2 GCGTATCTTT TTTTAAATT TGTGTTTTT GGTAGTAAT TATATTCCAA ATCAGCTTGT AATTCCTTG TATGCCGCTT TGAAGG ATTGCTAAT
ODC1 AGCATAATGT TCTATTGTC TCTATTATAT CGCTTAAATA GTTATTAAAA CTGTGATATA AATTGTATCC TATCTGCAAC CCTCTGAGT CTCTGATAG

1566                                     Poly A site                                     1665
ODC2 TGTGATTTTC TCTAATATG AACTTTTAA AATTAGTTTA AGAACAATA TGGGTAAGA GTTTGTGGG TCATGATATT TGTGTGACTA TAAAGCATC
ODC1 GTAGTTATGT TGTGTTGCC TACCAGCATC ATAATATTC TGTCTGAGA TAAAGCCAGT TAGCCATCCA GCTTTTGGT AAGGATTTAA TCACATATGT

```

Figure 12

19 / 21

	1		50
N. tabacum cv Xanthi	I	GASPTA	G
N. tabacum cv BY2	I	GASPTA	G
N. tabacum cv SC58	V	GASPTA	G
D. stramonium	A	TP	R
L. esculentum	A	PV	K
S. cerevisiae	MSSTQVGNALSSSTTTLVDL	NSTVTQKKYYKD	ETLHNLLLELK
H. sapiens	MNF	GN	
	51		100
N. tabacum cv Xanthi	L		D
N. tabacum cv BY2	L		D
N. tabacum cv SC58	L		D
D. stramonium	V		D
L. esculentum	V		E
S. cerevisiae	LELLHEQAHPKIFQ	KARIGRNNETCDPG	ENS FIC
H. sapiens	EEFDCH--FLDEGFT	KILDQKNEVSSS	DADA
	101		150
N. tabacum cv Xanthi	KS	I	
N. tabacum cv BY2	KS	I	
N. tabacum cv SC58	KS	I	
D. stramonium	NAG		
L. esculentum	NS		
S. cerevisiae	NAVKE	DTKV	LAELEV
H. sapiens	RLK	RVT	DSKAIVKT
	151		200
N. tabacum cv Xanthi	V	Y	Y
N. tabacum cv BY2	V	Y	Y
N. tabacum cv SC58	V	Y	Y
D. stramonium	V	Y	Y
L. esculentum	E	I	Y
S. cerevisiae	VAF	FRY	SKN
H. sapiens	P	E	I
	201		250
N. tabacum cv Xanthi	L	D	A
N. tabacum cv BY2	L	D	A
N. tabacum cv SC58	L	D	A
D. stramonium	L	D	A
L. esculentum	L	D	A
S. cerevisiae	-ATD	STQ	RLST
H. sapiens	-ATD	SKV	RLSV
	251		300
N. tabacum cv Xanthi	N	E	KL
N. tabacum cv BY2	N	E	KL
N. tabacum cv SC58	N	E	KL
D. stramonium	N	E	KL
L. esculentum	N	E	KL
S. cerevisiae	S	FTSLYK	VRD
H. sapiens	T	PETFVQ	SD
	301		350
N. tabacum cv Xanthi	AV	K	KQ
N. tabacum cv BY2	AV	K	KQ

Figure 13 (a)

Figure 13 (b)

21 / 21

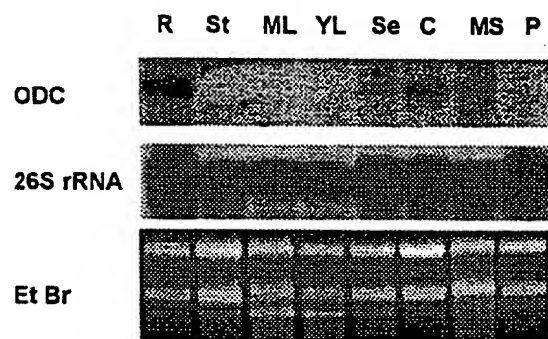
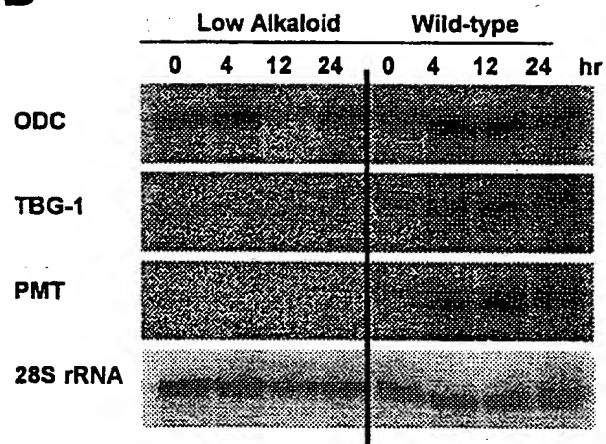
A**B**

Figure 14

SEQUENCE LISTING

<110> Timko P, Michael

<120> Regulation of Gene Expression in Tobacco for
Manipulation of Plant Growth and Secondary Metabolism

<130> 4981*239

<140>

<141>

<160> 26

<170> PatentIn Ver. 2.0

<210> 1

<211> 1120

<212> DNA

<213> Plant

<400> 1

```

ctgagttgac aagaacaatt cctggtgaat cagatggatg aagataatag aggtgggtgg 60
aatctataac caaagcagct ggttgagtga ctgtgagagt tgcagaaaca attgaagggt 120
catttggtgga atttggggcc atttcaaagg aaaaagaaaa gatgacttag cattaataaa 180
tcaaattaaa ataaggctta gcgttaaaat caaaggaaat ggcaagcctg gctcctggag 240
caatgcttct gaggacagta gtaaaaacaa tatcagacaa aaagtaaagt tgtattattt 300
agcttgagga taaagtatgt cattagtttt gtgagagatt tgggtgtcctc tacaatgatt 360
gttgaagtcc ctatttatag ctatacacag gaaacaaaat cctaggatca agccccctctt 420
aaatgacaat aatgggggta atgatgaata tgtagcggca tgacatgaat gccaaaattc 480
tccgcaacga ctatttattt aatattgagg aatatttttt attaaatact atctggtgac 540
aagcattcgt ttgcttcgtg tgattacgtt gattttggga tctactctat accaaccgaa 600
gccgttgtcc ttgatcttcg ctttcattta attcatcttc cgtctgcctc cgatttcaca 660
agtcatgcac ccattcaatt atttaatgga aaccaatttt accctataca aatggtacat 720
cattcgtcaa atactttact tggatataaa caattttgcc cgaggagtaa acagatgcga 780
agaaagaaaag cagacgatta aagaaatttt taaaaaagga gagagaaatg aacacacaca 840
tgtactaata aaattagggt actactttac taataattgg acagagacta aattcatatt 900
ttagttccaa aatgtctcgg gcagtccaac catgcacgtt gtaatgattt tttaactcta 960
ttatatcgag ttgcgccctc cactcctcgg tgtccaaatt gtatataaat gcatatgtgt 1020
ctattgggag tgtacatcaa gctttcataa agtacaaatc gtaatacttg ttgaaacata 1080
atactttctc ttctccaatt tgtttagttt aattttgaaa 1120

```

<210> 2

<211> 3091

<212> DNA

<213> Plant

<400> 2

```

ctgagttgac aagaacaatt cctggtgaat cagatggatg aagataatag aggtgggtgg 60
aatctataac caaagcagct ggttgagtga ctgtgcgagt tgcagaaaca attgaagggt 120
catttggtgga atttggggcc atttcaaagg aaaaagaaaa gatgacttag cattaataaa 180
tcaaattaaa ataaggctta gcgttaaaat caaaggaaat ggcaagcctg gctcctggag 240
caatgcttct gaggacagta gtaaaaacaa tatcagacaa aaagtaaagt tgtattattt 300
agcttgagga taaagtatgt cattagtttt gtgagagatt tgggtgcctc tacaatgatt 360
gttgaagtcc ctatttatag ctatacacag gaaacaaaat cctaggatca agccctctt 420
aaatgacaat aatgggggta atgatgaata tgtagcggca tgacatgaat gccaaaattc 480
tccgcaacga ctatttattt aatattgagg aatatttttt attaaatact atctggtgac 540
aagcattcgt ttgcttccgt tgattacggt gattttggga tctactctat accaaccgaa 600
gccgttgctc ttgatcttcg ctttcattta attcatcttc cgtctgcctc cgatttcaca 660
agtcattgac ccattcaatt atttaatgga aaccaatttt accctataca aatggtacat 720
cattcgtcaa atactttact tggatataaa caattttgcc cgaggagtaa acagatgcga 780
agaaagaaag cagacgatta aagaaatttt taaaaagga gagagaaatg aacacacaca 840
tgtactaata aaattagggg actactttac taataattgg acagagacta aattcatatt 900
ttagttccaa aatgtctcgg gcagtcacac catgcacggt gtaatgattt tttactcta 960
ttatatcgag ttgcgccctc cactcctcgg tgtccaaatt gtatataaat gcatatgtgt 1020
ctattgggag tgtacatcaa gctttcataa agtacaaatc gtaatacttg ttgaaacata 1080
atactttctc ttctccaatt tgtttagttt aattttgaaa atggaagtca tatctaccaa 1140
cacaaatggc tctaccatct tcaagagtgg tgccattccc atgaatggcc accataatgg 1200
cacttccaaa caccaaaacg gccacaagaa tgggacttcc gaacaacaga acgggacaat 1260
cagccttgat aatggcaacg agctactggg aaactccaat tgtattaagc ctggttggtt 1320
ttcagagttt agcgcattat ggccagggtt gtactgagaa agaaactcaa atgcataatt 1380
aaagttaaaa ttgttaggct aatataagga gttgatattc ttttagtgat taattaaaaa 1440
ggaaaaagta tcaataaat tcaaaaaatg gatagtaact tcgcatatta ctctacacat 1500
taatttgaaa taaatcgaat tttgcagggt aagcattctc acttaagggt gagaagttac 1560
tggtccaggg gaagtctgac taccaagatg tcatgctctt tgaggtaaat aatattttta 1620
tacacatgct tccattttaa ttgatacttt taatttactt ttactttatt gcatgtgtac 1680
gtacagtcag caacttatgg gaaggttctg actttggatg gagcaattca acacacagag 1740
aatggtggat ttccatacac tgaaatgatt gttcatcttc cacttggttc catcccaaac 1800
ccaaaaaagg ttttgatcat cggcggagga attggtttta cattattoga aatgcttcgt 1860
tactctacaa tcgaaaaaat tgacattggt gagatcgatg acgtggtagt tgatgtaagt 1920
caaacttctt ttactcacat aaaaaaatgg tttagattgc ttcttggtat ttttctaaaa 1980
gaatactatt tttttaaacc aaaattttct tttttacagg tatctagaaa atttttccct 2040
tatctcgctg ctaattttta cgatectcgt gtaaccctag tccttgagga tgggtgcgtat 2100
ttgataatct cgcttttggt ttatctttta tttttattgc atttaatttt taccttttgg 2160
tgtgtgggta attcacctgc cattggttct ctttcatttc aggggctgca tttgtaaagg 2220
ctgcacaagc agaataattat gatgctatta tagtggactc ttctgatccc attggtactc 2280
tattacttct taataccaag actaatctta ttgaataagc tactaataaa cggttaattga 2340
tttctaaaaa aatataattt cagggtccagc aaaagatttg tttgagaggc cattctttga 2400
ggcagtagct aaagccctaa ggccaggagg agttgtatgc acacaggctg aaagcatttg 2460
gcttcatatg catattatta agcaaatcat tgctaactgt cgtcaagtct ttaagggtc 2520
tgtcaactat gcttggacta ctgttccaac atatccaacg tatttttctc tctctctctc 2580
ttcctataaa attggaaggt ttgattctat aattgtcaag aaatggagaa tcagttccaa 2640
gaaaaaccaa cttcttttct tttactcttc aagggtattgt gtttaatttt ttttcaactg 2700
atatgatcaa ttattttgat ttcagcgggt tgattgggta tatgctctgc tctactgaag 2760
gaccagaaat tgacttcaag aatccagtaa atccaattga caaagagaca gctcaagtca 2820
agtccaaatt agcacctctc aagttctaca actctgatgt aacttcatat ctcacaattt 2880

```

cttttttccct attgtacttt atgtttcttcg tcaaatttta taattaactc ttttcaaatt 2940
 gtctttttttt ttttcagatt cacaaagcag cattcatttt gccatctttc gccagaagta 3000
 tgatcgagtc ttaatcaact gattaatgaa tactggtggt acaatcattg gaccaagatc 3060
 aataagtgaag agacgtattg tatgagaatt c 3091

<210> 3
 <211> 353
 <212> PRT
 <213> Plant

<400> 3

Met Glu Val Ile Ser Thr Asn Thr Asn Gly Ser Thr Ile Phe Lys Ser
 1 5 10 15

Gly Ala Ile Pro Met Asn Gly His His Asn Gly Thr Ser Lys His Gln
 20 25 30

Asn Gly His Lys Asn Gly Thr Ser Glu Gln Gln Asn Gly Thr Ile Ser
 35 40 45

Leu Asp Asn Gly Asn Glu Leu Leu Gly Asn Ser Asn Cys Ile Lys Pro
 50 55 60

Gly Trp Phe Ser Glu Phe Ser Ala Leu Trp Pro Gly Glu Ala Phe Ser
 65 70 75 80

Leu Lys Val Glu Lys Leu Leu Phe Gln Gly Lys Ser Asp Tyr Gln Asp
 85 90 95

Val Met Leu Phe Glu Ser Ala Thr Tyr Gly Lys Val Leu Thr Leu Asp
 100 105 110

Gly Ala Ile Gln His Thr Glu Asn Gly Gly Phe Pro Tyr Thr Glu Met
 115 120 125

Ile Val His Leu Pro Leu Gly Ser Ile Pro Asn Pro Lys Lys Val Leu
 130 135 140

Ile Ile Gly Gly Gly Ile Gly Phe Thr Leu Phe Glu Met Leu Arg Tyr
 145 150 155 160

Pro Thr Ile Glu Lys Ile Asp Ile Val Glu Ile Asp Asp Val Val Val
 165 170 175

Asp Val Ser Arg Lys Phe Phe Pro Tyr Leu Ala Ala Asn Phe Asn Asp
 180 185 190

Pro Arg Val Thr Leu Val Leu Gly Asp Gly Ala Ala Phe Val Lys Ala

195	200	205
Ala Gln Ala Glu Tyr Tyr Asp Ala Ile Ile Val Asp Ser Ser Asp Pro		
210	215	220
Ile Gly Pro Ala Lys Asp Leu Phe Glu Arg Pro Phe Phe Glu Ala Val		
225	230	235 240
Ala Lys Ala Leu Arg Pro Gly Gly Val Val Cys Thr Gln Ala Glu Ser		
	245	250 255
Ile Trp Leu His Met His Ile Ile Lys Gln Ile Ile Ala Asn Cys Arg		
	260	265 270
Gln Val Phe Lys Gly Ser Val Asn Tyr Ala Trp Thr Thr Val Pro Thr		
	275	280 285
Tyr Pro Thr Gly Val Ile Gly Tyr Met Leu Cys Ser Thr Glu Gly Pro		
	290	295 300
Glu Ile Asp Phe Lys Asn Pro Val Asn Pro Ile Asp Lys Glu Thr Ala		
305	310	315 320
Gln Val Lys Ser Lys Leu Ala Pro Leu Lys Phe Tyr Asn Ser Asp Ile		
	325	330 335
His Lys Ala Ala Phe Ile Leu Pro Ser Phe Ala Arg Ser Met Ile Glu		
	340	345 350

Ser

<210> 4

<211> 711

<212> DNA

<213> Plant

<400> 4

```

gaattcaatg gagaaggaaa atatttccag tgtaaacaca agtgaatgaa gagaagccaa 60
aataatctct atcattcaag ccttaggtgg agattaaaaa aattatttac tttcttatca 120
aagtaatagg tgatcaacag ctttcgtaaa acgtcattag gagaatatta taatctcttt 180
tatgctgaag aaccacata aggaagatca taaaatacat gactttcaga tgacttcttg 240
gagctttatt tttaaagagt ggctagctgg tcagcaaaga ggtgctcgtc agatatcata 300
aaattttact attatttgtt ttaagagggg gatggggcac acatgcttgt gacaaaagta 360
agaggaagaa aggagacaga agaggaaata gatttggggg gggggggggg ggtttcacia 420
tcaaagaaaa tttttaaaat ggagagagaa atgagcacac acatatacta acaaaathtt 480
actaataatt gcaccgagac aaacttatat ttagttcca aaatgtcagt ctaaccctgc 540

```

```

acgttgtaat gaatttttaa ctattatatt atatcgagtt gcgccctcca ctccctcggtg 600
tccaaattgt attttaaagc atagatgttt attgggagtg tacagcaagc ttccggaaaa 660
tacaaaccat aatactttct cttcttcaat ttgtttagtt taattttgaa a 711

```

<210> 5

<211> 3129

<212> DNA

<213> Plant

<400> 5

```

gaattcaatg gagaaggaaa atatttccag tgtaaacaca agtgaatgaa gagaagccaa 60
aataatctct atcattcaag ccttaggtgg agattaaaaa aattattttac tttcttatca 120
aagtaatagg tgatcaacag ctttcgtaaa acgtcattag gagaatatta taatctcttt 180
tatgctgaag aaccacata aggaagatca taaaatacat gactttcaga tgacttcttg 240
gagctttatt tttaaagagt ggctagctgg tcagcaaaga ggtgctcgtc agatatcata 300
aaattttact attatttgtt ttaagaggga gatggggcac acatgcttgt gacaaaagta 360
agaggaagaa aggagacaga agaggaaata gatttggggg gggggggggg ggtttcacaa 420
tcaaagaaaa tttttaaaat ggagagagaa atgagcacac acatatacta acaaaatttt 480
actaataatt gcaccgagac aaacttatat ttagttcca aaatgtcagt ctaaccctgc 540
acgttgtaat gaatttttaa ctattatatt atatcgagtt gcgccctcca ctccctcggtg 600
tccaaattgt attttaaagc atagatgttt attgggagtg tacagcaagc ttccggaaaa 660
tacaaaccat aatactttct cttcttcaat ttgtttagtt taattttgaa aatggaagtc 720
atatctacca acacaaatgg ctctaccatc ttcaagaatg gtgccattcc catgaacggc 780
caccaaaatg gcacttctga acacctcaac ggctaccaga atggcacttc caaacaccaa 840
aacgggcacc agaatggcac tttcgaacat cggaacggcc accagaatgg gacatccgaa 900
caacagaacg ggacaatcag ccatgacaat ggcaacgagc tactgggaag ctccgactct 960
attaagcctg gctggttttc agagtttagc gcattatggc caggttagta ctaagaaagc 1020
aactcaaatg catcggcctc ttgttgctac taaatataga gagctatcat acttttaggg 1080
actaactaaa aaggaaagat tatcacaggg acgaagtga cagttaactt cgcatattat 1140
cagacgcatt aatttgaaat aatcgaattt tgcaggtgaa gcattctcac ttaagggttg 1200
gaagtacta ttccagggga agtctgatta ccaagatgtc atgctctttg aggtaattaa 1260
tattctaata cacatgcttt aatttaaagt gatactttta atttactttt agtttattgc 1320
atgtgcacgt acagtcagca acttatggga aggttctgac tttggatgga gcaattcaac 1380
atacagagaa tgggtgattt ccatacactg aaatgattgt tcatctacca cttgggttcca 1440
tcccaaacc ccaaaaagggt ttgatcatcg gcggaggaat tggttttaca ttattcgaaa 1500
tgcttcgtta tccttcaatc gaaaaaattg acattgttga gatcgatgac gtggtagttg 1560
atgtaagtca aacttctttt acccacataa agaaaatgat ttagattgca attcttttta 1620
tttttctaaa agaataaata tatttctttt ttttttttta aaacaaaatt ctctttctta 1680
caggtatcca gaaaattttt cccttatctg gcagctaatt ttaacgatcc tcgtgtaacc 1740
ctagttctcg gagatggtgc gtatatgata gtctcgtttt atattttatt tcacttgatt 1800
tttacctttt tttgtggtta attaatacat taccattggt tctctttacc ttcaggagct 1860
gcatttgtaa aggtgcaca agcgggatat tatgatgcta ttatagtga ctcttctgat 1920
cccattggtg cgctattact atttaatacc aagactattc ttattaaata agctactaag 1980
aaactaattg aataattaat aaacgtaact gtaattgatt tctaaaataa tatatataat 2040
ttcaggtcca gcaaaagatt tggttgagag gccattcttt gaggcagtag ccaaagccct 2100
taggccagga ggagttgtat gcacacaggc tgaaagcatt tggcttcata tgcatattat 2160
taagcaaatc attgctaact gtctcaagt ctttaagggt tctgtcaact atgcttgac 2220
aaccgttcca acatatccca cgtattcttt ttctctctct ctcttctgt ctttttcgat 2280

```

```

gcaatgtaaa tttataaaat tggaagtccg ttttactttt ctatagacgt agatcctaaa 2340
attgtcaaga aatggagaat tgacttacaa gaaaaatcaa cttcttttca tttactattc 2400
tttttggtga caaactttac ttattatttc gttctaaaat gaaaatttat ttttatattt 2460
taaaataatt tagctttaaa cttttaattt tacttggtat atttttaata aaaaagattt 2520
atagtcaaat aaatgttggt accatataaa aacctccgca tttttaagat cataagtttc 2580
agagtcaaac gagttaattt attttttagt tgccggtgcg gagtcaaatt atgtcataaa 2640
aattgaaacg gagtgagaac atttttattt cgagtaaact ttcaaggat tgtgtttaat 2700
ttcaagtgat actgatcaat gatgtcttaa atattttgat ttcagcgggtg tgatcggtta 2760
tatgtctctgc tctactgaag ggccagaagt tgacttcaag aatccagtaa atccaattga 2820
caaagagaca actcaagtca agtccaaatt aggacctctc aagttctaca actctgatgt 2880
aacttcatat ctcacaattt ctttttccgt tttactgtat gttcttcgtc aaattttata 2940
actaactcct ttcataattgt cttttttttc agattcacia agcagcattc attttaccat 3000
ctttcgccag aagtatgata gagtcttaat caagtgaata atgaacactg gtagtacaat 3060
cattggacca agatcgagtc ttaatcaagt gaataaataa gtgaaatgcy acgtattgta 3120
ggagaattc                                     3129

```

<210> 6
 <211> 375
 <212> PRT
 <213> Plant

<400> 6

```

Met Glu Val Ile Ser Thr Asn Thr Asn Gly Ser Thr Ile Phe Lys Asn
  1             5             10             15

```

```

Gly Ala Ile Pro Met Asn Gly His Gln Asn Gly Thr Ser Glu His Leu
          20             25             30

```

```

Asn Gly Tyr Gln Asn Gly Thr Ser Lys His Gln Asn Gly His Gln Asn
          35             40             45

```

```

Gly Thr Phe Glu His Arg Asn Gly His Gln Asn Gly Thr Ser Glu Gln
          50             55             60

```

```

Gln Asn Gly Thr Ile Ser His Asp Asn Gly Asn Glu Leu Leu Gly Ser
          65             70             75             80

```

```

Ser Asp Ser Ile Lys Pro Gly Trp Phe Ser Glu Phe Ser Ala Leu Trp
          85             90             95

```

```

Pro Gly Glu Ala Phe Ser Leu Lys Val Glu Lys Leu Leu Phe Gln Gly
          100            105            110

```

```

Lys Ser Asp Tyr Gln Asp Val Met Leu Phe Glu Ser Ala Thr Tyr Gly
          115            120            125

```

```

Lys Val Leu Thr Leu Asp Gly Ala Ile Gln His Thr Glu Asn Gly Gly
          130            135            140

```

Phe Pro Tyr Thr Glu Met Ile Val His Leu Pro Leu Gly Ser Ile Pro
 145 150 155 160

Asn Pro Lys Lys Val Leu Ile Ile Gly Gly Gly Ile Gly Phe Thr Leu
 165 170 175

Phe Glu Met Leu Arg Tyr Pro Ser Ile Glu Lys Ile Asp Ile Val Glu
 180 185 190

Ile Asp Asp Val Val Val Asp Val Ser Arg Lys Phe Phe Pro Tyr Leu
 195 200 205

Ala Ala Asn Phe Asn Asp Pro Arg Val Thr Leu Val Leu Gly Asp Gly
 210 215 220

Ala Ala Phe Val Lys Ala Ala Gln Ala Gly Tyr Tyr Asp Ala Ile Ile
 225 230 235 240

Val Asp Ser Ser Asp Pro Ile Gly Pro Ala Lys Asp Leu Phe Glu Arg
 245 250 255

Pro Phe Phe Glu Ala Val Ala Lys Ala Leu Arg Pro Gly Gly Val Val
 260 265 270

Cys Thr Gln Ala Glu Ser Ile Trp Leu His Met His Ile Ile Lys Gln
 275 280 285

Ile Ile Ala Asn Cys Arg Gln Val Phe Lys Gly Ser Val Asn Tyr Ala
 290 295 300

Trp Thr Thr Val Pro Thr Tyr Pro Thr Gly Val Ile Gly Tyr Met Leu
 305 310 315 320

Cys Ser Thr Glu Gly Pro Glu Val Asp Phe Lys Asn Pro Val Asn Pro
 325 330 335

Ile Asp Lys Glu Thr Thr Gln Val Lys Ser Lys Leu Gly Pro Leu Lys
 340 345 350

Phe Tyr Asn Ser Asp Ile His Lys Ala Ala Phe Ile Leu Pro Ser Phe
 355 360 365

Ala Arg Ser Met Ile Glu Ser
 370 375

<210> 7

<211> 1134

<212> DNA

<213> Plant

<400> 7

```

gctgtacaaa aggatgtctc aaatcatttg gaatattaat tctgcaatca acaagaaata 60
ccccactatt aagaccatt atcactggca caaaaattat gagatcatta aacatcttaa 120
acctgtccct atttggaaga gtgtggtatg ggagatgcct cccagggagt acctaaagct 180
gaatactgat ggaagtttta acaaacaaat tgggaaagca gggattggag ggattctcag 240
agatgaagag ggaggctttg tcatggcttt ttcatgcct ataactctata ataacatcag 300
tgaagcagaa ttgaaagcca tcaagtatgg gtgtgaatgg tgcaaataca aaggaatatc 360
aaacttcatt gtggaaactg actcgaggat gatctatgac atactacaga ccaaaaatct 420
aagcaacaac aagttgaaac aagagaccga gaaattaatg gagattctgg acacctgcag 480
gacacctgtt acccattgcc ttccggaagc aaatcaagtg gcagactggt ttgctaaaga 540
ggccaccaga gctaacgaag gtatcactca tacagatttt agacaggtat caaaagcggc 600
caagggccct ttcttcattg atatgtggca ggtcccttat tttagaatta gatatgaaaa 660
atctaatttt tttttgtaag ttaattctgt gtatagttag aggaaatcgt ctaatatgta 720
tttttgccca tagactcttc ctctccttag gtaaaaaggt agctccgagg taaggtttat 780
gttccctca gtgtaacctt tttttgttta tataatagac atgggtatggg tccagctaaa 840
cccccaacac cacaggggat agatacctgg gtgattgggt tattttttta aaaaaaaac 900
tttactaata attgcacgga gacaaaactt atattttagt tccaaaatga cagtccaacc 960
atgcacgttg taatgatttt ttaactctat tatatcgagt tccgccctcc actcctcggt 1020
gtccaaattg tatttaaattg catagatatg tttattggga gtgtacatca agctttcaga 1080
aaatacaaac cataatactt tctcttctcc aatttgctta gtttaatttg gaaa 1134

```

<210> 8

<211> 3269

<212> DNA

<213> Plant

<400> 8

```

gctgtacaaa aggatgtctc aaatcatttg gaatattaat tctgcaatca acaagaaata 60
ccccactatt aagaccatt atcactggca caaaaattat gagatcatta aacatcttaa 120
acctgtccct atttggaaga gtgtggtatg ggagatgcct cccagggagt acctaaagct 180
gaatactgat ggaagtttta acaaacaaat tgggaaagca gggattggag ggattctcag 240
agatgaagag ggaggctttg tcatggcttt ttcatgcct ataactctata ataacatcag 300
tgaagcagaa ttgaaagcca tcaagtatgg gtgtgaatgg tgcaaataca aaggaatatc 360
aaacttcatt gtggaaactg actcgaggat gatctatgac atactacaga ccaaaaatct 420
aagcaacaac aagttgaaac aagagaccga gaaattaatg gagattctgg acacctgcag 480
gacacctgtt acccattgcc ttccggaagc aaatcaagtg gcagactggt ttgctaaaga 540
ggccaccaga gctaacgaag gtatcactca tacagatttt agacaggtat caaaagcggc 600
caagggccct ttcttcattg atatgtggca ggtcccttat tttagaatta gatatgaaaa 660
atctaatttt tttttgtaag ttaattctgt gtatagttag aggaaatcgt ctaatatgta 720
tttttgccca tagactcttc ctctccttag gtaaaaaggt agctccgagg taaggtttat 780
gttccctca gtgtaacctt tttttgttta tataatagac atgggtatggg tccagctaaa 840
cccccaacac cacaggggat agatacctgg gtgattgggt tattttttta aaaaaaaac 900
tttactaata attgcacgga gacaaaactt atattttagt tccaaaatga cagtccaacc 960
atgcacgttg taatgatttt ttaactctat tatatcgagt tccgccctcc actcctcggt 1020

```

```

gtccaaattg tatttaaattg catagatatg tttattggga gtgtacatca agcttttcaga 1080
aaatacaaac cataataactt tctcttctcc aatttgctta gtttaatttg gaaaatggaa 1140
gtcatatcta ccaacacaaa tggctctact atcttcaaga atgggtgccat tcccatgaac 1200
ggttaccaga atggcacttc caaacaccaa aacggccacc agaatggcac ttccgaacat 1260
cggaacggcc accagaatgg gatttccgaa caccaaaacg gccaccagaa tggcacttcc 1320
gagcatcaga acggccatca gaatgggaca atcagccatg acaacggcaa cgagctacag 1380
ctactgggaa gctccaactc tattaagcct gggtggtttt cagagtttag cgcattatgg 1440
ccaggttagt actaagaaag aaactcaaat gcctcgtact cttgtattct gctttgcgta 1500
taatttagat gatggtggtt gactaagcac tgagttaaaa aataaaaagt ttaaagttaa 1560
attgttacta tagagagcta tatctttagg aactaactaa aaaggaaaaa ttatcacata 1620
aaattgggat gaagtaagca gtttaacttc catattattc gacacattaa tttgaaataa 1680
atcgaatttt gcaggtgag cattctcact taagggtgag aagtactat tccaggggaa 1740
gtctgattac caagatgtca tgctctttga ggtaattaat taataactaa agtcaagctc 1800
atgtatgatt atatttaaag tggatTTTT cgtttatttt taatttattg cacgtgtacg 1860
tacagtacgc aacatatggg aaggttctga ctttggatgg agcaattcaa cacacagaga 1920
atgggtggatt tccatacact gaaatgattg ttcactctcc acttgggtcc atcccaaac 1980
ctaaaaaggt tttgatcatc ggcgaggaa tgggttttac attattcgaa atgcttcggt 2040
atcctacaat cgaaaaaatt gacattgttg agatcgatga cgtggtagtt gatgtaagtc 2100
aaacttcttt tactcacata aaaaaatgat ttagattctt atttttctaa aagaattaaa 2160
acaaaatttt ccgttttaca ggtatctaga aaatttttcc cttatcttgc tgctaatttt 2220
agcgatcctc gtgtaaccct agtcttggga gatggtgcgt atttgataat ctcgttttta 2280
ttttatcttt tacttttatt ttatttaatt tttacctttt tgtgtgtggt taattcacct 2340
gccattgggt ctttttattt caggggctgc atttgtaaag gccgcacaag caggatatta 2400
tgatgctatt atagtggact cttctgatcc cattgggtact ctattactac ttaataccaa 2460
gactattctt attaaataag ctactaataa acgtaactct gatagttttc taaaataata 2520
taatttcagg tccagcaaaa gacttgtttg agaggccatt ctttgaggca gtagccaaag 2580
ccctaaggcc aggaggagt gtatgcacac aggctgaaag catttggtt catatgcata 2640
ttattaagca aatcattgct aactgtcgtc aagtctttaa gggctctgtc aactatgctt 2700
ggactactgt tccaacatat ccaacgtatt tttctctctc tcttcctata aaattggaag 2760
ttttgattct ataattgtca agaaatggag aatcagttcc aagaaaaacc aaattctttt 2820
cttttactct tcaagggtgt ttaaagtttt ttaaactgat actgatcaat tattttgatt 2880
tcagcgggtg gattggttat atgctctggt ctactgaagg accagaagtt gacttcaaga 2940
atccagtaaa tccaattgac aaagagacaa ctcaagtcaa gtccaaatta gcacctctca 3000
agttctacaa ctctgatgta acttcatatc tcaatttctt ttttcttatt gtactttatg 3060
ttcttagtca aattttataa ttaactcttt tcaaattgtc ttttttttcc agattcacaa 3120
agcagcattc attttgccat ctttcgccag aagtatgata gagtcttaat caagtgacta 3180
atgaatactg gcgttacaat cattggacca agatcgagtc ttaatcaagt gaataaataa 3240
gtgaaatgcg acgtattgta taagaattc 3269

```

<210> 9

<211> 381

<212> PRT

<213> Plant

<400> 9

Met Glu Val Ile Ser Thr Asn Thr Asn Gly Ser Thr Ile Phe Lys Asn

1

5

10

15

Gly Ala Ile Pro Met Asn Gly Tyr Gln Asn Gly Thr Ser Lys His Gln
 20 25 30
 Asn Gly His Gln Asn Gly Thr Ser Glu His Arg Asn Gly His Gln Asn
 35 40 45
 Gly Ile Ser Glu His Gln Asn Gly His Gln Asn Gly Thr Ser Glu His
 50 55 60
 Gln Asn Gly His Gln Asn Gly Thr Ile Ser His Asp Asn Gly Asn Glu
 65 70 75 80
 Leu Gln Leu Leu Gly Ser Ser Asn Ser Ile Lys Pro Gly Trp Phe Ser
 85 90 95
 Glu Phe Ser Ala Leu Trp Pro Gly Glu Ala Phe Ser Leu Lys Val Glu
 100 105 110
 Lys Leu Leu Phe Gln Gly Lys Ser Asp Tyr Gln Asp Val Met Leu Phe
 115 120 125
 Glu Ser Ala Thr Tyr Gly Lys Val Leu Thr Leu Asp Gly Ala Ile Gln
 130 135 140
 His Thr Glu Asn Gly Gly Phe Pro Tyr Thr Glu Met Ile Val His Leu
 145 150 155 160
 Pro Leu Gly Ser Ile Pro Asn Pro Lys Lys Val Leu Ile Ile Gly Gly
 165 170 175
 Gly Ile Gly Phe Thr Leu Phe Glu Met Leu Arg Tyr Pro Thr Ile Glu
 180 185 190
 Lys Ile Asp Ile Val Glu Ile Asp Asp Val Val Val Asp Val Ser Arg
 195 200 205
 Lys Phe Phe Pro Tyr Leu Ala Ala Asn Phe Ser Asp Pro Arg Val Thr
 210 215 220
 Leu Val Leu Gly Asp Gly Ala Ala Phe Val Lys Ala Ala Gln Ala Gly
 225 230 235 240
 Tyr Tyr Asp Ala Ile Ile Val Asp Ser Ser Asp Pro Ile Gly Pro Ala
 245 250 255
 Lys Asp Leu Phe Glu Arg Pro Phe Phe Glu Ala Val Ala Lys Ala Leu
 260 265 270

Arg Pro Gly Gly Val Val Cys Thr Gln Ala Glu Ser Ile Trp Leu His
 275 280 285

Met His Ile Ile Lys Gln Ile Ile Ala Asn Cys Arg Gln Val Phe Lys
 290 295 300

Gly Ser Val Asn Tyr Ala Trp Thr Thr Val Pro Thr Tyr Pro Thr Gly
 305 310 315 320

Val Ile Gly Tyr Met Leu Cys Ser Thr Glu Gly Pro Glu Val Asp Phe
 325 330 335

Lys Asn Pro Val Asn Pro Ile Asp Lys Glu Thr Thr Gln Val Lys Ser
 340 345 350

Lys Leu Ala Pro Leu Lys Phe Tyr Asn Ser Asp Ile His Lys Ala Ala
 355 360 365

Phe Ile Leu Pro Ser Phe Ala Arg Ser Met Ile Glu Ser
 370 375 380

<210> 10
 <211> 469
 <212> DNA
 <213> Plant

<400> 10
 gtcgacctct gattccacaa gtcatgcacc cattcaatta tttaatggaa accaatttta 60
 ccctgtacaa atggtacaaa tactttcctt ggataaaaac aattttgcct aaggagtaaa 120
 cagatgcgaa gtaagaaagc agacgactaa agaaaatttt aaaaaaggag agagaaatga 180
 gcacacacac gtactaataa aattagggta ctactttact aataattgga cagagactaa 240
 attcatattt tagttccaaa atgtctcggg cagtccaacc atgcacgttg taatgagttt 300
 ttaactctat tatctcgagt tgcgcctcc actcctctgt gtccaagttg tatataaatg 360
 catatatgtc tattgggagt gtacagcgag ctttcataaa gtacaaatca taatacttgt 420
 tgaaacataa tactttctct tctccaattt gtttagttta attttgaaa 469

<210> 11
 <211> 3001
 <212> DNA
 <213> Plant

<400> 11
 gtcgacctct gattccacaa gtcatgcacc cattcaatta tttaatggaa accaatttta 60
 ccctgtacaa atggtacaaa tactttcctt ggataaaaac aattttgcct aaggagtaaa 120
 cagatgcgaa gtaagaaagc agacgactaa agaaaatttt aaaaaaggag agagaaatga 180
 gcacacacac gtactaataa aattagggta ctactttact aataattgga cagagactaa 240
 attcatattt tagttccaaa atgtctcggg cagtccaacc atgcacgttg taatgagttt 300

```

ttaactctat tatctcgagt tgcgccctcc actcctctgt gtccaagttg tatataaatg 360
catatatgtc tattgggagt gtacagcgag ctttcataaa gtacaaatca taatacttgt 420
tgaacataaa tactttctct tctccaattt gtttagttta attttgaaaa tggaagtcac 480
atctaccaac acaaatggct cgaccatctt caagaatggg gccattccca tgaatggcca 540
ccagagtggc acttccaaac acctcaacgg ctaccagaac ggcacttcca aacacaaaaa 600
cggccaccat aatggcactt ccgaacatcg gaacggccac cagaatggga tttccgaaca 660
ccaaaacggc caccagaatg ggacttccga acatcggaac ggccaccaga atgggatttc 720
cgaacaccaa aacggccacc agaatgggac ttccgaacac caaaacggcc accagaatgg 780
gacttccgaa caacagaacg ggacaatcag ccatgacaat ggcaacgagc tactgggaaa 840
ctccaactct attaagcttg gttggttttc agagtttagc gcattatggc caggtttagt 900
ctgagaaaga aactcaaatt catatttaaa gttaaaattg ttaggctaata ataagaagtt 960
gattttcttt tagtgattaa ttaaaaaagg aaagagtatc aaataaattc caaaaaatga 1020
ccagtaactt cgcatattat tctacacatt aatttgaaat aaatcgaatt ttgcagggtg 1080
agcattctcc cttaagggtg agaagttact atttcagggg aagtctgact accaagatgt 1140
catgctcttt gaggtaaata atattctaata acacatgctt taatatgaat aaatactttt 1200
aatttacttt tagtttattg cacgtgtacg tacagtcagc aacatatggg aaggttttga 1260
ctttggatgg agcaattcaa cacacagaga atgggtggatt tccatacact gaaatgattg 1320
ttcatcttcc acttgggtcc atcccaaacc caaaaaagggt tttgatcatc ggcggaggaa 1380
ttggttttac attattcgaa atgcttcgtt atcctacaat cgaaaaaatt gacattgttg 1440
aaatcgatga cgtggttagt gatgtaagtc aaatttcttt tactcacata aaaaaatgat 1500
ttagattgct tctttttatt tttctaaaag aataaatata ttctctctta gttttaaaca 1560
aaattctctt tcttacaggt atctagaaaa tctttccctt atctcgcagc taattttaat 1620
gatcctcgtg taaccctcgt tctcggagat ggtgcgtatt tataatctcg tttttgtttt 1680
atcttttatt tttatttcat ttaatttacc tttttgtgtg tggttaattt acccgtcatt 1740
ggttctcttt catttcaggg gctgcatttg taaaggctgc acaagcagga tattatgatg 1800
ctattatagt ggactcttct gatcccatg gtactctatt actacttaat accaagacta 1860
atcttattga ataagctact aataaactgt aattgatttc taaaataata taatttcagg 1920
tccagcaaaa gatttgtttg agaggccatt ctttgaggca gtagccaaag ccctaaggcc 1980
aggaggagtt gtatgcacac aggcggaaa gatttggtt catatgcata ttattaagca 2040
aatcattgct aactgtcgtc aagtctttta gggctctgtc aactacgctt ggactactgt 2100
tccaacatat ccacgtatt tctctctct ctctcttcat ctttgaaaat tgaaaatcct 2160
gactactttc ctctctttga ttctcgggt aaaggggctg agatcataag attttcaaga 2220
aatagataat gacgtccaag aaaaactaac ttcttttcat ttactattct ttttggtgac 2280
aaactttatt taattatttcg ttctaaagag aaaatttatt tttatatttt aaaataattt 2340
tgttttaaac ttttattttt acttattata tctttaataa aaaaattata gtcaaataaa 2400
tattatggcc aactaaaca tccaagtttt tgaaccata agtttttagag ccaaatgagt 2460
taatttgttt ttggtatgcg ggtgcggagt caaattatgt cacaataatt gtaatggagt 2520
gagcaaatth ttatttcgag taaactttca aggtattgtg ttaaagtttt ttcaactgat 2580
actaatcaat tatgtctcaa ccattttgat ttcagtgggt taattgggta tatgctctgc 2640
tctactgaag ggccagaagt tgacttcaag aatccaataa atccaattga caaagagaca 2700
actcaagtca agtccaaatt agcacctctc aagttttaca attctgatgt aacttcatat 2760
ctaacaattt ctttttctgt tttactgtat cttcattgtc aaaattttat aattaactct 2820
tctcaaatta tcttttttt tagattcaca aagcagcatt cattttgccca tctttcgcca 2880
gaagtatgat cgagtcctta tcaagtgaat aatgaacact ggtggtgcaa tcattggacc 2940
aagatcgagt cttaatcaag tgaataaata agtgaaatgc cgacgtattg tatgagaatt 3000
c 3001

```

<210> 12

<211> 419
 <212> PRT
 <213> Plant

<400> 12

Met Glu Val Ile Ser Thr Asn Thr Asn Gly Ser Thr Ile Phe Lys Asn
 1 5 10 15

Gly Ala Ile Pro Met Asn Gly His Gln Ser Gly Thr Ser Lys His Leu
 20 25 30

Asn Gly Tyr Gln Asn Gly Thr Ser Lys His Gln Asn Gly His His Asn
 35 40 45

Gly Thr Ser Glu His Arg Asn Gly His Gln Asn Gly Ile Ser Glu His
 50 55 60

Gln Asn Gly His Gln Asn Gly Thr Ser Glu His Arg Asn Gly His Gln
 65 70 75 80

Asn Gly Ile Ser Glu His Gln Asn Gly His Gln Asn Gly Thr Ser Glu
 85 90 95

His Gln Asn Gly His Gln Asn Gly Thr Ser Glu Gln Gln Asn Gly Thr
 100 105 110

Ile Ser His Asp Asn Gly Asn Glu Leu Leu Gly Asn Ser Asn Ser Ile
 115 120 125

Lys Leu Gly Trp Phe Ser Glu Phe Ser Ala Leu Trp Pro Gly Glu Ala
 130 135 140

Phe Ser Leu Lys Val Glu Lys Leu Leu Phe Gln Gly Lys Ser Asp Tyr
 145 150 155 160

Gln Asp Val Met Leu Phe Glu Ser Ala Thr Tyr Gly Lys Val Leu Thr
 165 170 175

Leu Asp Gly Ala Ile Gln His Thr Glu Asn Gly Gly Phe Pro Tyr Thr
 180 185 190

Glu Met Ile Val His Leu Pro Leu Gly Ser Ile Pro Asn Pro Lys Lys
 195 200 205

Val Leu Ile Ile Gly Gly Gly Ile Gly Phe Thr Leu Phe Glu Met Leu
 210 215 220

Arg Tyr Pro Thr Ile Glu Lys Ile Asp Ile Val Glu Ile Asp Asp Val

225 230 235 240
 Val Val Asp Val Ser Arg Lys Ser Phe Pro Tyr Leu Ala Ala Asn Phe
 245 250 255
 Asn Asp Pro Arg Val Thr Leu Val Leu Gly Asp Gly Ala Ala Phe Val
 260 265 270
 Lys Ala Ala Gln Ala Gly Tyr Tyr Asp Ala Ile Ile Val Asp Ser Ser
 275 280 285
 Asp Pro Ile Gly Pro Ala Lys Asp Leu Phe Glu Arg Pro Phe Phe Glu
 290 295 300
 Ala Val Ala Lys Ala Leu Arg Pro Gly Gly Val Val Cys Thr Gln Ala
 305 310 315 320
 Glu Ser Ile Trp Leu His Met His Ile Ile Lys Gln Ile Ile Ala Asn
 325 330 335
 Cys Arg Gln Val Phe Lys Gly Ser Val Asn Tyr Ala Trp Thr Thr Val
 340 345 350
 Pro Thr Tyr Pro Thr Gly Val Ile Gly Tyr Met Leu Cys Ser Thr Glu
 355 360 365
 Gly Pro Glu Val Asp Phe Lys Asn Pro Ile Asn Pro Ile Asp Lys Glu
 370 375 380
 Thr Thr Gln Val Lys Ser Lys Leu Ala Pro Leu Lys Phe Tyr Asn Ser
 385 390 395 400
 Asp Ile His Lys Ala Ala Phe Ile Leu Pro Ser Phe Ala Arg Ser Met
 405 410 415
 Ile Glu Ser

<210> 13

<211> 1636

<212> DNA

<213> Plant

<400> 13

ggcacgagat cagatccaat tctcttctgt gcttccttc tctgctetca aattcttcag 60
 atctacaaag tttcttctcat tttcagaggg cagacatgga aactttcttg ttcacctcag 120
 agtcagtcaa tgaaggccac cccgacaagc tctgcgacca ggtctcggat gcaattcttg 180

```

atgcttgctt agaacaggat ccagaaagca aggttgcatt tgaaacctgc acaaagacaa 240
acatggttat ggtctttgga gagatcacaa ccaaggccac tgttgactat gagaagatag 300
tgcgtgacac atgcagaggc attgggttca cctcagcaga tgttggcctt gacgctgaca 360
actgcaaggt tcttgtcaac atcgagcagc agagccctga cattgcccaa ggtgttcacg 420
gtcatcttac caagaaacca gaagagattg gagctggtga ccaaggtcac atgtttggct 480
atgccactga tgaaacccca gagctcatgc cccttaccga tgtttgggcc actaagcttg 540
gtgccaagct taccgaagtg aggaagaaca agacttgccc atggctcaga ccagatggca 600
agaccaagt tactgttgag tacaagaacg acaatggtgc catggtccca attagagttc 660
acactgttct catctcaact caacatgacg aaactgtcac aaacgaccag attgcccagg 720
acttgaaaga gcatgtgatc aaacctgtga tcccatctca gtaccttgat gagaatacca 780
tcttccacct caacccatca ggtcgttcg tcacgggtgg accacacgga gatgctggac 840
ttaccggcag gaaaattatc attgacacct acggaggctg ggggtgccat ggaggaggtg 900
ctttctcagg aaaggacctt actaagggtg acaggagtgg tgcttatatt gttagacagg 960
cagcaaagag tgtggtcgcc tcaggacttg ctgcgcgtg tattgtgcag gtttcttatg 1020
ctatcgggtg ggctgaacca ctttccgtgt ttgtgacac ttacaagact ggaacaattc 1080
cagacaagga tttttgact ctgatcaagg agaactttga cttcaggcct ggaatgatgt 1140
caatcaacct tgacttgta agaggaggca acttcaggta ccagaagact gcagcttatg 1200
gtcactttgg ccgtgatgac cccgacttct catgggagac tgtcaaggtc ctcaagccaa 1260
aagcttaagt gaggtgtagc cttttggcca ttatttttct tgcagaccaa taaacaagct 1320
tcatcatatc atgcattggt ggcaggagaa gagaatttgt gtctccattg gaggattcta 1380
tgagctctga gtcattgaac attgttattt ttctttcttt ttttttcacc cttttctgca 1440
gtaccttatt tttattttgt tactgttaag tagcagtgat ttaagttttc cctgttaagt 1500
agcagtgatt taagttttcc ctgttaagta gctggaatta agtttccatg ttctatcata 1560
ttatatgtga acttgtcaat tatctcctga ggtgaaagag tccttcaggg aatagtttaa 1620
aaaaaaaaa aaaaaa 1636

```

<210> 14

<211> 390

<212> PRT

<213> Plant

<400> 14

```

Met Glu Thr Phe Leu Phe Thr Ser Glu Ser Val Asn Glu Gly His Pro
  1             5             10             15

```

```

Asp Lys Leu Cys Asp Gln Val Ser Asp Ala Ile Leu Asp Ala Cys Leu
      20             25             30

```

```

Glu Gln Asp Pro Glu Ser Lys Val Ala Cys Glu Thr Cys Thr Lys Thr
      35             40             45

```

```

Asn Met Val Met Val Phe Gly Glu Ile Thr Thr Lys Ala Thr Val Asp
      50             55             60

```

```

Tyr Glu Lys Ile Val Arg Asp Thr Cys Arg Gly Ile Gly Phe Thr Ser
      65             70             75             80

```

```

Ala Asp Val Gly Leu Asp Ala Asp Asn Cys Lys Val Leu Val Asn Ile

```

85										90					95				
Glu	Gln	Gln	Ser	Pro	Asp	Ile	Ala	Gln	Gly	Val	His	Gly	His	Leu	Thr				
			100						105				110						
Lys	Lys	Pro	Glu	Glu	Ile	Gly	Ala	Gly	Asp	Gln	Gly	His	Met	Phe	Gly				
		115					120					125							
Tyr	Ala	Thr	Asp	Glu	Thr	Pro	Glu	Leu	Met	Pro	Leu	Thr	His	Val	Trp				
	130					135					140								
Ala	Thr	Lys	Leu	Gly	Ala	Lys	Leu	Thr	Glu	Val	Arg	Lys	Asn	Lys	Thr				
145					150				155					160					
Cys	Pro	Trp	Leu	Arg	Pro	Asp	Gly	Lys	Thr	Gln	Val	Thr	Val	Glu	Tyr				
			165					170						175					
Lys	Asn	Asp	Asn	Gly	Ala	Met	Val	Pro	Ile	Arg	Val	His	Thr	Val	Leu				
			180					185					190						
Ile	Ser	Thr	Gln	His	Asp	Glu	Thr	Val	Thr	Asn	Asp	Gln	Ile	Ala	Gln				
	195						200					205							
Asp	Leu	Lys	Glu	His	Val	Ile	Lys	Pro	Val	Ile	Pro	Ser	Gln	Tyr	Leu				
	210						215				220								
Asp	Glu	Asn	Thr	Ile	Phe	His	Leu	Asn	Pro	Ser	Gly	Arg	Phe	Val	Ile				
225					230				235					240					
Gly	Gly	Pro	His	Gly	Asp	Ala	Gly	Leu	Thr	Gly	Arg	Lys	Ile	Ile	Ile				
			245					250					255						
Asp	Thr	Tyr	Gly	Gly	Trp	Gly	Ala	His	Gly	Gly	Gly	Ala	Phe	Ser	Gly				
		260					265						270						
Lys	Asp	Pro	Thr	Lys	Val	Asp	Arg	Ser	Gly	Ala	Tyr	Ile	Val	Arg	Gln				
		275					280					285							
Ala	Ala	Lys	Ser	Val	Val	Ala	Ser	Gly	Leu	Ala	Arg	Arg	Cys	Ile	Val				
	290					295					300								
Gln	Val	Ser	Tyr	Ala	Ile	Gly	Val	Ala	Glu	Pro	Leu	Ser	Val	Phe	Val				
305					310				315				320						
Asp	Thr	Tyr	Lys	Thr	Gly	Thr	Ile	Pro	Asp	Lys	Asp	Ile	Leu	Thr	Leu				
			325					330				335							
Ile	Lys	Glu	Asn	Phe	Asp	Phe	Arg	Pro	Gly	Met	Met	Ser	Ile	Asn	Leu				

340

345

350

Asp Leu Leu Arg Gly Gly Asn Phe Arg Tyr Gln Lys Thr Ala Ala Tyr
 355 360 365

Gly His Phe Gly Arg Asp Asp Pro Asp Phe Ser Trp Glu Thr Val Lys
 370 375 380

Val Leu Lys Pro Lys Ala
 385 390

<210> 15
 <211> 1596
 <212> DNA
 <213> Plant

<400> 15
 ggcacgaggg gaacaagaga aacatcatat tattgaatcc ctagtttctt ttctttccct 60
 ttgattcctt cctctcattt acctctctct tttcttccct tgtttggatg gccggccaaa 120
 caatcatcgt ttccgggttg aaccggcgcc ccattcttca gtccacaatt ggccggcgag 180
 cttctcttac agcggcgccg gggcgggaaa acggcaccag aaaagtcac cctctctcaa 240
 gagatgcctt acaagatttc atgttatcaa tcataaccca aaaattacaa gatgagaaac 300
 aaccttttta cgtgctagac ttgggtgagg ttgtttctct tatggacca tggaatctg 360
 ctctcccaaa tatccgtcca ttttacgctg ttaaagttaa ccctgaaccg tcgttccttt 420
 caattttatc tgctatgggc tcaaattttg attgtgctag ccgagctgaa attgagtatg 480
 ttttatctct tggcatttca cctgaccgta ttgttttcgc aaatccatgc aaaccggaat 540
 ccgatattat ttttgacgca aaagtggggg tgaatcttac aacctatgat tctgaagacg 600
 aggttttaca gatccgaaag catcaccgga aatccgaact cttgctccgc atcaagccca 660
 tgctcgacgg caacgcgaga tgcccaatgg gcccgaaata cggcgcgctt ccagaagaag 720
 tcgaccgct gctccgggca gctcaagccg ccggtctcac cgtatccggc gtctcattcc 780
 acatcggtag cggagatgcc gattcaaacg cttatctcgg cgccatagcc gcggctaagg 840
 aagtgtttga aacagctgct aaactcggga tgtcgaaaat gactgttcta gacgtcggcg 900
 gcggttttac atccggccac cagttcacia ccgccgccgt cgccgttaaa tcagctttta 960
 aacaacactt cgatgacgaa ccggagtga caatcatagc tgaaccgggt cggttttttg 1020
 cagagacggc gtttactttg gcaacgacga ttataggga aagagtgagg ggtgaattga 1080
 gggagtattg gattaacgac gggctgtacg gttcgatgaa ctgtgtactt tacgaccatg 1140
 cgacggtgaa tgcaacgccg ttagctgttc tgtcgaaatc tagtaacgtt acctgcggcg 1200
 ggtcgaaaac gtttccgacg actgtgtttg ggcccacttg tgatgctctt gatactgttt 1260
 taagggatta ccagttaccg gagctgcagg ttaatgattg gctggttttt cctaatatgg 1320
 gtgcttatac taaagctgct gggccaatt ttaatggatt taatacttcc gccattgtta 1380
 ctacacctgc ttattcttat ccaagctgat gaaccacctg tattaggaat tactaccgtg 1440
 gttttgatgg ttttttccct ttttgggtat ctttttttta attttgttgt ttttggtagt 1500
 aatttatatt ccaaatcagc ttgtaattct cttgtatgcc ataagaatgc aaggatttgc 1560
 taattgtgat tttctctaaa aaaaaaaaaa aaaaaa 1596

<210> 16
 <211> 433

<212> PRT

<213> Plant

<400> 16

Met Ala Gly Gln Thr Ile Ile Val Ser Gly Leu Asn Pro Ala Ala Ile
 1 5 10 15

Leu Gln Ser Thr Ile Gly Gly Gly Ala Ser Pro Thr Ala Ala Ala Ala
 20 25 30

Ala Glu Asn Gly Thr Arg Lys Val Ile Pro Leu Ser Arg Asp Ala Leu
 35 40 45

Gln Asp Phe Met Leu Ser Ile Ile Thr Gln Lys Leu Gln Asp Glu Lys
 50 55 60

Gln Pro Phe Tyr Val Leu Asp Leu Gly Glu Val Val Ser Leu Met Asp
 65 70 75 80

Gln Trp Lys Ser Ala Leu Pro Asn Ile Arg Pro Phe Tyr Ala Val Lys
 85 90 95

Cys Asn Pro Glu Pro Ser Phe Leu Ser Ile Leu Ser Ala Met Gly Ser
 100 105 110

Asn Phe Asp Cys Ala Ser Arg Ala Glu Ile Glu Tyr Val Leu Ser Leu
 115 120 125

Gly Ile Ser Pro Asp Arg Ile Val Phe Ala Asn Pro Cys Lys Pro Glu
 130 135 140

Ser Asp Ile Ile Phe Ala Ala Lys Val Gly Val Asn Leu Thr Thr Tyr
 145 150 155 160

Asp Ser Glu Asp Glu Val Tyr Lys Ile Arg Lys His His Pro Lys Ser
 165 170 175

Glu Leu Leu Leu Arg Ile Lys Pro Met Leu Asp Gly Asn Ala Arg Cys
 180 185 190

Pro Met Gly Pro Lys Tyr Gly Ala Leu Pro Glu Glu Val Asp Pro Leu
 195 200 205

Leu Arg Ala Ala Gln Ala Ala Arg Leu Thr Val Ser Gly Val Ser Phe
 210 215 220

His Ile Gly Ser Gly Asp Ala Asp Ser Asn Ala Tyr Leu Gly Ala Ile
 225 230 235 240

Ala Ala Ala Lys Glu Val Phe Glu Thr Ala Ala Lys Leu Gly Met Ser
 245 250 255

Lys Met Thr Val Leu Asp Val Gly Gly Gly Phe Thr Ser Gly His Gln
 260 265 270

Phe Thr Thr Ala Ala Val Ala Val Lys Ser Ala Leu Lys Gln His Phe
 275 280 285

Asp Asp Glu Pro Glu Leu Thr Ile Ile Ala Glu Pro Gly Arg Phe Phe
 290 295 300

Ala Glu Thr Ala Phe Thr Leu Ala Thr Thr Ile Ile Gly Lys Arg Val
 305 310 315 320

Arg Gly Glu Leu Arg Glu Tyr Trp Ile Asn Asp Gly Leu Tyr Gly Ser
 325 330 335

Met Asn Cys Val Leu Tyr Asp His Ala Thr Val Asn Ala Thr Pro Leu
 340 345 350

Ala Val Leu Ser Asn Arg Ser Asn Val Thr Cys Gly Gly Ser Lys Thr
 355 360 365

Phe Pro Thr Thr Val Phe Gly Pro Thr Cys Asp Ala Leu Asp Thr Val
 370 375 380

Leu Arg Asp Tyr Gln Leu Pro Glu Leu Gln Val Asn Asp Trp Leu Val
 385 390 395 400

Phe Pro Asn Met Gly Ala Tyr Thr Lys Ala Ala Gly Ser Asn Phe Asn
 405 410 415

Gly Phe Asn Thr Ser Ala Ile Val Thr His Leu Ala Tyr Ser Tyr Pro
 420 425 430

Ser

<210> 17

<211> 2074

<212> DNA

<213> Plant

<400> 17

tggttaactgg accgacgcga catttgctcgt atatgtctta atcgggctag tcgctgacaa 60

```

catcatccac caagtcaaag ttcggaaatt catatcgttt ctcacatct tctatccgag 120
aatgagggg actatctgta tacgggtcaaa accgagtcgt ccttcatat gactaatcga 180
gattagaaca taatggtcta aggttcacat ttataataac gagccatgat atagagttag 240
gttgtcaagc tcaagcccca gagagcgatc aatatcgaga tcgagccaag gttaaactcg 300
agacctagag accgatcaat accgagaccg accaagtcaa ggtcgagctc gagaccaga 360
gaccggtcaa gattgagatc ggccaagatc gagatcgagc caagaaatta aaaagtcggt 420
atagccgcat ttagggagag aatctctgcg gaaatcacga cttgaatcag ggaaaaacta 480
attaattaat ctatcatgtg atccccacta tgtattttta attatactca aaatgggatt 540
ccccactat attaagagtg gttatcattt gtaatggaga gacatacaca cattcattct 600
gacatatata gaaaatagag caaatactat ccttttttgg cttttgatat ttagtcatat 660
tgtttcttct acccattgtt cttcactcaa tttggaggtg ataaaacttg aagggtttaag 720
ctaactagtc cattcggtt gcattcattt cttttacaat aatttcgtca tcatttattt 780
atcttctcaa ttgtactaag ttataccacg ttttttaga actgcgtata aattcaactc 840
tatccatttt tcgggtaaac accgaatata tagcacaata gcaccctcaa ttgcaaaagt 900
ccaaagccaa ggggttcattc ctttctgaag aaatgagata gagaattgaa aatctaattt 960
agttatctaa atctttataa tttagccttc catataagaa aaaggaaaca aattaactga 1020
agaacaatag cctcgcatag atttaccttc tccatataaa ttttgtttat actcaatttt 1080
tttgcaaatg tgtctaaaat gataggactt gcaaattttt atttaacatt tcctactcct 1140
ctttaatttt caagaaatta attttaagca ttctcgattt gctctgcccg ctccgtcccg 1200
ttgccatctc tgactcggtt aggacctcga ttgcaaaaat ccaaacccaa ggaaccttcc 1260
atacattaca taagccacaa aatagtaact attaaaaact accaatatat cctcaaatac 1320
tcgcgattat ttcataccta acacgtttac cttatcttct cgtaatgacg ctacattagt 1380
tagtgatata aaataccgaa tttaccacgc ggcaaccctc cgtgtcttat ccacggcccg 1440
agagaatctc ttagccccc aaatacgaaa attaacttct agaattttat tttctgggta 1500
ttaccatgaa aataaagaaa aagagaaaag tcaagaaatt taattgggct aatactggg 1560
tccactgcc agccacgcat ttccctccta tataaagcgt cgtcacctct catgcaaatc 1620
tcgctcactt cacagttgtt agtttcacgt tctcttctca attcccataa aagaaaccct 1680
tccgttaggt tccgtccta tttctcttc ttctacgctt cctcttctga tatcaatatc 1740
tgtatggtgt tttcttgtt cgaatttttag atttgtttg ctttaatac ctgtaacctt 1800
ataattctct gtttaaacca aaaacttagc ttcttctgaa gtcaggggtg ggatatttg 1860
atcgtgtaag agtggttag aaggtgatta tcttttgatt cagttccttt tttgcttctt 1920
ttgagggggt agcgggggc tcggcctcgg cgggttttaa tagcccccac ctattacaac 1980
cattgggcaa aaacatcatt aaatctgtac aaagcaaac ctaatttag ttaattttc 2040
tgtattcttt gattctttaa cagaagaaga agag

```

2074

<210> 18

<211> 4321

<212> DNA

<213> Plant

<400> 18

```

tggttaactgg accgacgca catttgctgt atatgtctta atcggttag tcgctgacaa 60
catcatccac caagtcaaag ttcggaaatt catatcgttt ctcacatct tctatccgag 120
aatgagggg actatctgta tacgggtcaaa accgagtcgt ccttcatat gactaatcga 180
gattagaaca taatggtcta aggttcacat ttataataac gagccatgat atagagttag 240
gttgtcaagc tcaagcccca gagagcgatc aatatcgaga tcgagccaag gttaaactcg 300
agacctagag accgatcaat accgagaccg accaagtcaa ggtcgagctc gagaccaga 360
gaccggtcaa gattgagatc ggccaagatc gagatcgagc caagaaatta aaaagtcggt 420

```

atagccgcat ttagggagag aatctctgcg gaaatcacga cttgaatcag ggaaaaacta 480
 attaattaat ctatcatgtg atccccacta tgtattttta attatactca aaatgggatt 540
 cccccactat attaagagtg gttatcattt gtaatggaga gacatacaca cattcattct 600
 gacatatata gaaaatagag caaatactat ccttttttgg cttttgatat ttagtcatat 660
 tgtttcttct acccattgtt cttcactcaa tttggaggtg ataaaacttg aagggtttaag 720
 ctaactagtc cattcgggtt gcattcattt cttttacaat aatttcgtca tcatttattt 780
 attttctcaa ttgtactaag ttataccacg tatttttaga actgcgata aattcaactc 840
 tatccatttt tcgggtaaac accgaataca tagcacaata gcaccctcaa ttgcaaaagt 900
 ccaaagccaa ggggttcattc ctttctgaag aatgagata gagaattgaa aatctaattt 960
 agttatctaa atctttataa ttttagccttc catataagaa aaaggaaaca aattaactga 1020
 agaacaatag cctcgcatag atttaccttc tccatataaa ttttgtttat actcaatttt 1080
 tttgcaaatg tgtctaaaat gataggactt gcaaattttt atttaacatt tcctactcct 1140
 ctttaatttt caagaaatta attttaagca ttctcgattt gctctgcccg ctccgtcccg 1200
 ttgccatctc tgactcggat aggacctcga ttgcaaaaat ccaaacccaa ggaaccttcc 1260
 atacattaca taagccacaa aatagtaact attaaaaact accaatatat cctcaaatac 1320
 tcgcgattat ttcataccta acacgtttac cttatcttct cgtaatgacg ctacattagt 1380
 tagtgatata aaataccgaa tttaccacgc ggcaaccctc cgctgtctat ccacggcccg 1440
 agagaatctc ttagccccc aaatacgaaa attaaacttct agaattttat tttctgggta 1500
 ttaccatgaa aataaagaaa aagagaaaag tcaagaaatt taattgggct aatactgggg 1560
 tccactgcc agccacgcat ttccctcta tataaagcgt cgtcacctct catgcaaatc 1620
 tcgctcactt cacagttgtt agtttcacgt tctcttctca attcccataa aagaaacct 1680
 tccgttaggt tccgctcta ttttctctt tctacgctt cctcttctga tatcaatata 1740
 tgtatgggtt ttttctgtt cgaattttag atttgttttg cctttaatac ctgtaacctt 1800
 ataattctct gtttaaacca aaaacttagc ttcttctgaa gtcaggggtg ggatatttg 1860
 atcgtgtaag agtggttag aagggtgatta tcttttgatt cagttccttt tttgcttctt 1920
 ttgagggggg agccggggcc tcggcctcgg cgggttttaa tagcccccct ctattacaac 1980
 cattgggcaa aaacatcatt aaatctgtac aaagcaaacc ctttaatttag ttttaatttt 2040
 tgtattcttt gattctttaa cagaagaaga agagatgccg gccctagggt gttgcgtaga 2100
 cgctactgtt tccctcctc tcggctatgc cttctctcgg gatagctctc ttcccgcgcc 2160
 ggagttcttt acctccggcg tacctcctac aaactccgcc gcgggttcca ttgggtctcc 2220
 ggatctgtcc tctgctttgt acggggtcga tgggtgggga gctccttatt tctccgttaa 2280
 ctctaaccga gatattctccg tccgaccaca tggtagcgac aactccccc accaggaaat 2340
 tgacctctc aaggtcgtga aaaaggcctc cgaccgaaa aattcagggg ggctcgggct 2400
 tcagctgcct cttgttggtt gcttccctga tgtgctaaaa aaccgggttg aatctctgca 2460
 atcggctttt gatctcgtg ttcattccca gggctatggg gccactacc aagggtggtta 2520
 tcccgtaaaa tgcaatcaag acaggttcgt ggtggaagat attgtcaaat tcgggtcgtc 2580
 attccgggtt cgggttggaag ctgggtctaa acccgagctc ctggttagcca tgagctgtct 2640
 ctgcaggggc agtgctgagg gccttctcgt ttgcaatggg ttcaaggacg ctgagtacat 2700
 ttcgcttgct ttggttgcaa gaaagctcat gttaaacact gtaattgttc ttgaacaaga 2760
 ggaggagctt gacctgtga ttgatataag ccgtaagatg gctgttcggc ccgtaattgg 2820
 acttcgggct aagctcagga ccaagcattc aggccatttt ggatccactt ctggagaaaa 2880
 aggtaagttt gggcttacia cgacccaaat tgttcgtgta gtgaagaagc tggaagaatc 2940
 cggaatgctg gattgccttc agttgctgca ttttcacatt ggatctcaga tcccttcaac 3000
 ggcgttgctt gctgatggtg ttggtgagge tgctcagatt tattgtgaat taatccgtct 3060
 tgggtcgggt atgaagttca ttgatactgg aggtgggctc ggaattgatt atgatgggtac 3120
 taaatcatgt gattcagatg tctctgttgg ctatggcatt caagaatacg cctccacagt 3180
 tgtccaggcg gttcaatatg tttgcgaccg taagggcggtg aagcaccag tgatttgagc 3240
 cgaaagtggc agggcaattg tttctcatca ctcaattctg attttcgaag ccgtgtctgc 3300

```

ttctagtcac tcatgttctt cttcacatct gtcttctggt ggcctccaat ccatggcgga 3360
gacgctcaat gaagatgccc ttgctgatta ccgcaattta tctgctgctg cagttcgtgg 3420
agagtacgag acgtgtgtac ttactctga tcagttgaaa cagagatgtg tggatcagtt 3480
taaagaaggg tccttgggta ttgaacatct tgctgctggt gatagcatct gtgattttgt 3540
atcaaaggct atgggggctg ctgatcctat ccgcacttac catgtgaatc tgtcaatttt 3600
cacttcaatt cctgattttt gggccttttg tcaattgttt ccgattgttc caatacaccg 3660
tttagatgaa aagcctgcag taaggggaat attatcggac ttgacttggt acagtgatgg 3720
gaaggttgat aagttcattg gtggcgaatc aagcttgacg ctgcatgaat tgggaagtaa 3780
tgggcgatggt ggtgggtatt atctggggat gtttttgggt ggggcttatg aggaggcgct 3840
cggaggactc cacaacctgt ttggtggacc aagcgtggtg cgcgtggtgc agagcgatag 3900
cgctcacagc ttcgccatgt ctgctccgt cctggcccgc tcctgcgcgg acgtgctccg 3960
agcgatgcag cacgagcccg agctcatgtt cgagactctc aagcaccgtg cggaggaatt 4020
cttgaacaa gaagaagaca aagggtggc cattgcctt ttggccagca gcttagctca 4080
gtccttccat aacatgcctt acctgtggc gcctgcctt tgctgcttca ctgcagttac 4140
tgctaacaac ggtggctata actactatta cagtgatgag aatgcagcag attctgctac 4200
aggggaggat gagatttggc cctattgcac tgcttgaagt gttgtcgtag catctccagt 4260
tttagtttgt cgtcgaagtt gtctgtttt gaataatacc cttagtgtgt gatgtttttc 4320
t 4321

```

<210> 19

<211> 720

<212> PRT

<213> Plant

<400> 19

```

Met Pro Ala Leu Gly Cys Cys Val Asp Ala Thr Val Ser Pro Pro Leu
  1             5             10             15

```

```

Gly Tyr Ala Phe Ser Arg Asp Ser Ser Leu Pro Ala Pro Glu Phe Phe
          20             25             30

```

```

Thr Ser Gly Val Pro Pro Thr Asn Ser Ala Ala Gly Ser Ile Gly Ser
      35             40             45

```

```

Pro Asp Leu Ser Ser Ala Leu Tyr Gly Val Asp Gly Trp Gly Ala Pro
      50             55             60

```

```

Tyr Phe Ser Val Asn Ser Asn Gly Asp Ile Ser Val Arg Pro His Gly
      65             70             75             80

```

```

Thr Asp Thr Leu Pro His Gln Glu Ile Asp Leu Leu Lys Val Val Lys
          85             90             95

```

```

Lys Ala Ser Asp Pro Lys Asn Ser Gly Gly Leu Gly Leu Gln Leu Pro
      100             105             110

```

```

Leu Val Val Arg Phe Pro Asp Val Leu Lys Asn Arg Leu Glu Ser Leu
      115             120             125

```

Gln Ser Ala Phe Asp Leu Ala Val His Ser Gln Gly Tyr Gly Ala His
 130 135 140

Tyr Gln Gly Val Tyr Pro Val Lys Cys Asn Gln Asp Arg Phe Val Val
 145 150 155 160

Glu Asp Ile Val Lys Phe Gly Ser Ser Phe Arg Phe Gly Leu Glu Ala
 165 170 175

Gly Ser Lys Pro Glu Leu Leu Leu Ala Met Ser Cys Leu Cys Arg Gly
 180 185 190

Ser Ala Glu Gly Leu Leu Val Cys Asn Gly Phe Lys Asp Ala Glu Tyr
 195 200 205

Ile Ser Leu Ala Leu Val Ala Arg Lys Leu Met Leu Asn Thr Val Ile
 210 215 220

Val Leu Glu Gln Glu Glu Glu Leu Asp Leu Val Ile Asp Ile Ser Arg
 225 230 235 240

Lys Met Ala Val Arg Pro Val Ile Gly Leu Arg Ala Lys Leu Arg Thr
 245 250 255

Lys His Ser Gly His Phe Gly Ser Thr Ser Gly Glu Lys Gly Lys Phe
 260 265 270

Gly Leu Thr Thr Thr Gln Ile Val Arg Val Val Lys Lys Leu Glu Glu
 275 280 285

Ser Gly Met Leu Asp Cys Leu Gln Leu Leu His Phe His Ile Gly Ser
 290 295 300

Gln Ile Pro Ser Thr Ala Leu Leu Ala Asp Gly Val Gly Glu Ala Ala
 305 310 315 320

Gln Ile Tyr Cys Glu Leu Ile Arg Leu Gly Ala Gly Met Lys Phe Ile
 325 330 335

Asp Thr Gly Gly Gly Leu Gly Ile Asp Tyr Asp Gly Thr Lys Ser Cys
 340 345 350

Asp Ser Asp Val Ser Val Gly Tyr Gly Ile Gln Glu Tyr Ala Ser Thr
 355 360 365

Val Val Gln Ala Val Gln Tyr Val Cys Asp Arg Lys Gly Val Lys His
 370 375 380

Pro Val Ile Cys Ser Glu Ser Gly Arg Ala Ile Val Ser His His Ser
 385 390 395 400
 Ile Leu Ile Phe Glu Ala Val Ser Ala Ser Ser His Ser Cys Ser Ser
 405 410 415
 Ser His Leu Ser Ser Gly Gly Leu Gln Ser Met Ala Glu Thr Leu Asn
 420 425 430
 Glu Asp Ala Leu Ala Asp Tyr Arg Asn Leu Ser Ala Ala Val Arg
 435 440 445
 Gly Glu Tyr Glu Thr Cys Val Leu Tyr Ser Asp Gln Leu Lys Gln Arg
 450 455 460
 Cys Val Asp Gln Phe Lys Glu Gly Ser Leu Gly Ile Glu His Leu Ala
 465 470 475 480
 Ala Val Asp Ser Ile Cys Asp Phe Val Ser Lys Ala Met Gly Ala Ala
 485 490 495
 Asp Pro Ile Arg Thr Tyr His Val Asn Leu Ser Ile Phe Thr Ser Ile
 500 505 510
 Pro Asp Phe Trp Ala Phe Gly Gln Leu Phe Pro Ile Val Pro Ile His
 515 520 525
 Arg Leu Asp Glu Lys Pro Ala Val Arg Gly Ile Leu Ser Asp Leu Thr
 530 535 540
 Cys Asp Ser Asp Gly Lys Val Asp Lys Phe Ile Gly Gly Glu Ser Ser
 545 550 555 560
 Leu Gln Leu His Glu Leu Gly Ser Asn Gly Asp Gly Gly Gly Tyr Tyr
 565 570 575
 Leu Gly Met Phe Leu Gly Gly Ala Tyr Glu Glu Ala Leu Gly Gly Leu
 580 585 590
 His Asn Leu Phe Gly Gly Pro Ser Val Val Arg Val Val Gln Ser Asp
 595 600 605
 Ser Ala His Ser Phe Ala Met Ser Arg Ser Val Pro Gly Pro Ser Cys
 610 615 620
 Ala Asp Val Leu Arg Ala Met Gln His Glu Pro Glu Leu Met Phe Glu
 625 630 635 640

Thr Leu Lys His Arg Ala Glu Glu Phe Leu Glu Gln Glu Glu Asp Lys
645 650 655

Gly Leu Ala Ile Ala Ser Leu Ala Ser Ser Leu Ala Gln Ser Phe His
660 665 670

Asn Met Pro Tyr Leu Val Ala Pro Ala Ser Cys Cys Phe Thr Ala Val
675 680 685

Thr Ala Asn Asn Gly Gly Tyr Asn Tyr Tyr Tyr Ser Asp Glu Asn Ala
690 695 700

Ala Asp Ser Ala Thr Gly Glu Asp Glu Ile Trp Ser Tyr Cys Thr Ala
705 710 715 720

<210> 20
<211> 2118
<212> DNA
<213> Plant

<400> 20
gaattcctta tccggatttc tggtagcgag actgtaatat ggagtcacat tctcctcgat 60
tcgggattaa aattaggtga cttgggacac cctaaatctc ccaagtggcg actctgaaat 120
aaataaacia atcccgtttc gattgtcctt aaattggaaa aaactccctt gtaccctccc 180
gggtacggaa aaaggagggtg tacagcaatg acccaaaact tttattgcta tacattttga 240
ggaatcaact tgatcaaaat ttatgggtga aattcaatgt ggtatgattt atattaggtc 300
ggacttttagc agatgtggtc acttcaattt gcggcacaaa taatgtacag ggataataat 360
aaaaagtact agaaatttga gtcataaagc tttttcaatt ttacaaaaga tattaagata 420
cttattaaat caaatgtact ttattaatgt aatagcatga aaaaacagcc tcatccgcct 480
gtcctcaccc cacaaaaagg agatagagaa aggaactaa tcttatttaa tttccacat 540
ataaaattta tctccttgta taaatcccca aaaaaaaata atcaatacta attattttta 600
tttaatcatc cgtataagaa agaagctaata taactgactt acaaaactgaa tagatagcac 660
aatagcactc tcaattacaa aaatccaaag ccgagggtca ttcctttcat caagaaatta 720
gatagggaat ggaaaatata atttaattat ctgaatcttt ataatttatc cttccatata 780
agaaaaagga aacaaattaa ctgaagagca tatagcctcg catagattta cttctccat 840
atgggtgggg aaaccgacaa accgcaccaa ttcgataatt cgagtcaaac tgaggaaaaa 900
aaaattcgac tatggttttg tttgatttgg tttattgttg ggataaaaaa tcgatcataa 960
ttggttttgt ttggttttaa ctaaagaaag tcaaaccgaa accaaacaaa cccgacatta 1020
catatataaa ttttttagat atatttaata tataaatata cttgttgtga tgtaatttat 1080
aaatatttct taaaaatatt cataatttta tcttttaaga tattatttcg taattagaac 1140
ttttgaatgt ttcttactcc tctttaattt gttagaaatt aatttgaagg agtttgaatt 1200
tgctccgccc ccgctccgtc ccgttgccat ccctgactca ataggataac agcaatctcg 1260
attgcaaaaa tccaaaccca aggaaccttc ccaacattac ataagctaca aagtagagta 1320

```

gtttattaaa taactaccaa tatatcctca aattctcgcg attatttcat acctaacacg 1380
cttaccttat cttctcgtaa tgacgtaca ttagttggtg atataaaata ccgaatttgc 1440
cacgcggcaa tcctccgctg tctatccacg gcccgagaga atctcttagc ccccaaaga 1500
tgaaaattaa cttctagaat tttattttct ggttattacc atgaaaataa ttaaataaaa 1560
aaaaagagaa aagtaaagat atttaattgg gctaaaactg ggggccacgg cccagccacg 1620
catttccctc ctatataaag cgtcgtcacc tctcatgcaa atctcgtca ctacacagtt 1680
gttagtttca cgttctcttc tcaattccca taacagaaac ccttccggtt ggtttccgtc 1740
ctatttttcc tcatcttctc cgtttctctt tctgaaatca atatctgtat ggtgtttttc 1800
ttgttcgaat ttttagattg ttttgtcttt aatactata acctaaatt ctctgtttta 1860
acaaaaact tagcttcttc tgaagtcagg gtggggattt ttggatcgtg taagagtgtg 1920
ttagagggtg attatctttt gattcagttc cttttttgct tcttttgagg gggtagccgg 1980
ggcctcggcc tcggcgggtt ttaatagccc ccacttatta caactattgg gcaaaaacat 2040
cattaaatct gtacaaaaca aaccctaat ttagtttaat tttctgtatt cattgatttt 2100
ttaacagaag aagaagag                                     2118

```

<210> 21

<211> 4368

<212> DNA

<213> Plant

<400> 21

```

gaattcctta tccggatttc tggtagcgag actgtaatat ggagtcattt tctcctcgat 60
tcgggattaa aattaggtga cttgggacac cctaaatctc ccaagtggcg actctgaaat 120
aaataaacia atcccgtttc gattgtcctt aaattggaaa aaactccctt gtaccctccc 180
gggtacggaa aaaggagggtg tacagcaatg acccaaaact tttattgcta tacattttga 240
ggaatcaact tgatcaaaat ttatgggtga aattcaatgt ggtatgattt atattaggtc 300
ggacttttagc agatgtggtc acttcaattt gcggaacaaa taatgtacag ggataataat 360
aaaaagtact agaaatttga gtcataaagc tttttcaatt ttacaaaaga tattaagata 420
cttattaaat caaatgtact ttattaatgt aatagcatga aaaaacagcc tcatccgcct 480
gtcctcacc cacaanaagg agatagagaa aggaactaa tcttatttaa ttttccacat 540
ataaaattta tatccttgta taaatcccca aaaaaaaaaa atcaatacta attattttta 600
tttaatcatc cgtataagaa agaagctaata taactgactt acaaaactgaa tagatagcac 660
aatagcactc tcaattacaa aaatccaaag ccgagggtca ttcctttcat caagaaatta 720
gatagggaat ggaaaatata atttaattat ctgaatcttt ataatttatc cttccatata 780
agaaaaagga aacaaattaa ctgaagagca tatagcctcg catagattta ccttctccat 840
atgggtgggg aaaccgacaa accgcaccaa ttcgataatt cgagtcaaac tgaggaaaaa 900
aaaattcgac tatggttttg tttgatttgg tttattgttg ggataaaaaa tcgatcataa 960
ttggttttgt ttggttttta ctaaaagaa tcaaaccgaa accaaacaaa cccgacatta 1020
catatataaa ttttttagat atatttaata tataaatata cttgttgtga tgtaatttat 1080
aaatatttct taaaaatatt cataatttta tcttttaaga tattatttcg tacttagaac 1140
ttttgaatgt ttcttactcc tctttaattt gttagaaatt aatttgaagg agtttgaatt 1200
tgetccgccc cgetccgtc cgttgccat ccctgactca ataggataac agcaatctcg 1260
attgcaaaaa tccaaacca aggaaccttc ccaacattac ataagctaca aagtagagta 1320
gtttattaaa taactaccaa tatatcctca aattctcgcg attatttcat acctaacacg 1380
cttaccttat cttctcgtaa tgacgtaca ttagttggtg atataaaata ccgaatttgc 1440
cacgcggcaa tcctccgctg tctatccacg gcccgagaga atctcttagc ccccaaaga 1500
tgaaaattaa cttctagaat tttattttct ggttattacc atgaaaataa ttaaataaaa 1560
aaaaagagaa aagtaaagat atttaattgg gctaaaactg ggggccacgg cccagccacg 1620

```

```

catttccctc ctatataaag cgctcgtcacc tctcatgcaa atctcgtcctca ctacacagtt 1680
gtagtattca cgttctcttc tcaattccca taacagaaac ccttccgtta ggtttccgtc 1740
ctatttttcc tcatcttctc cgtttcctct tctgaaatca atatctgtat ggtgtttttc 1800
ttgttcgaat ttttagatttg ttttgtcttt aatacctata accttaaatt ctctgtttta 1860
acaaaaaact tagcttcttc tgaagtcagg gtggggattt ttggatcgtg taagagtgtg 1920
ttagagggtg attatctttt gattcagttc cttttttgct tcttttgagg gggtagccgg 1980
ggcctcggcc tcggcggtt ttaatagccc ccatctatta caactattgg gcaaaaacat 2040
cattaaatct gtacaaaaca aacccttaat ttagtttaat tttctgtatt cattgatttt 2100
ttaacagaag aagaagagat gccggcccta ggtgtgtgtg tagatgctgc tgtgtttcc 2160
cctcctctca gctatgcctt ctctcgggat agctctcttc ccgcgcgga gttctttgcc 2220
tcggcgctac ctccgacaaa ttctgcgct gcttccattg ggtctccgga tttgtcgtct 2280
gctttatacg gggtcgatgg gtggggagct ccttatttct ctgttaactc taatggagat 2340
atctccgtcc gaccacacgg tacggacact ctccctcacc aggaattga cttctcaag 2400
gtcgtgaaaa aggcctccga ccgaaaaat tcagggtggc ttgggcttca gctgcctctt 2460
gtgttcgct tccctgatgt gttgaaaaac cggttggaat ctctgcaatc ggcttttgat 2520
ctcgcggttc attcccagg ctatggggcc cactaccaag gtgtttatcc cgtgaaatgc 2580
aatcaagaca ggttcgtggt ggaagatata gtgaaattcg ggtcgcatt ccggttcggg 2640
ttggaagccg ggtctaaacc cgagctcctg ttagccatga gctgtctctg caagggcagt 2700
gctgaggggc ttctcgtttg caatggtttc aaggacgtg agtacatttc gcttgctttg 2760
gttgcaagaa agctcatgtt aaacactgta attgtgcttg aacaagagga ggagcttgac 2820
cttgtgattg atataagcca taagatggct gtccggcctg taattggact tcgggctaag 2880
ctcaggacca agcattcagg ccattttgga tccactctg gagaaaaagg taagtttggg 2940
cttacaacga cccaaattgt tcgtgtggtg aagaagctag aagaatccgg aatgctggat 3000
tgtcttcagt tgctgcattt tcacattgga tctcagatcc cttctacggg gttgctagct 3060
gatggagttg gtgaggccgc tcagatttat tgtgaattag tccgtcttg agcgggtatg 3120
aagttcattg atattggagg tgggcttgga attgattatg atggtactaa atcatgcgat 3180
tctgatgtct ctggttgcta tggcattcaa gaatatgcct ccgcagttgt tcaggcggtt 3240
caatatgtat gcgaccgtaa ggtgtgaaa caccagtgta tctgcagcga aagtggcagg 3300
gcaattgttt ctcatcactc aattctgatt ttcgaagccg tctctgcttc tagtcaactca 3360
tgttcttctt cacatctgtc ttctggtggc ctccaatcca tggcgagac gctcaacgaa 3420
gatgcccttg ctgattaccg caatttatct gctgctgcag ttcgtggaga gtatgagaca 3480
tgtgtacttt actctgatca gttgaaacag agatgtgtgg atcagtttaa agaagggtcc 3540
ttgggtattg aacatcttgc tgctgttgat agcatctgtg attttgtatc aaaggctatg 3600
ggggctgctg atcctgtccg cacttaccat gtgaatctgt caattttcac ttcaattcct 3660
gatttttggt cctttggtca attgtttccg attgttccaa ttcaccgctt agatgaaaag 3720
cctgcagtga ggggaatatt atcggactta acttgtgaca gtgatgggaa ggttgataag 3780
ttcattggtg gcgaatcaag cttgccgcta catgaattgg gaagtaatgg cgatggtggt 3840
ggttattatc tggggatgtt ttgggtggg gcttatgagg aggcgctcgg aggactccac 3900
aacctgtttg gtggaccaag tgcgtgcgc gtggtgcaga gcgatagcgc tcacagcttt 3960
gccatgactc gctccgtccc tggccgtct tgctctgatg tgctccgagc gatgcagcac 4020
gagcccagac tcatgttcga gactctcaag caccgtgcgg aggaattctt ggaacaagaa 4080
gatgacaaag ggctggctgt tgaatctttg gccagcagcg tagctcagtc cttccataac 4140
atgccttacc ttgtggcgcc ttcattctgc cgcttactg ctgctactga taacaatggt 4200
ggctataatt actattacag tgatgagaat gcagcagatt ctgctacagg ggaggatgag 4260
atttggtcct attgcactgc ttgaagtgtt ctctagcat ctccagctt agtttgcgt 4320
cgaggttgct tgtttttgaa taataccctt agttggtgat gtttttct 4368

```

<210> 22

<211> 721
 <212> PRT
 <213> Plant

<400> 22

```

Met Pro Ala Leu Gly Cys Cys Val Asp Ala Ala Val Val Ser Pro Pro
  1              5              10              15

Leu Ser Tyr Ala Phe Ser Arg Asp Ser Ser Leu Pro Ala Pro Glu Phe
              20              25              30

Phe Ala Ser Gly Val Pro Pro Thr Asn Ser Ala Ala Ala Ser Ile Gly
      35              40              45

Ser Pro Asp Leu Ser Ser Ala Leu Tyr Gly Val Asp Gly Trp Gly Ala
      50              55              60

Pro Tyr Phe Ser Val Asn Ser Asn Gly Asp Ile Ser Val Arg Pro His
      65              70              75              80

Gly Thr Asp Thr Leu Pro His Gln Glu Ile Asp Leu Leu Lys Val Val
              85              90              95

Lys Lys Ala Ser Asp Pro Lys Asn Ser Gly Gly Leu Gly Leu Gln Leu
      100              105              110

Pro Leu Val Val Arg Phe Pro Asp Val Leu Lys Asn Arg Leu Glu Ser
      115              120              125

Leu Gln Ser Ala Phe Asp Leu Ala Val His Ser Gln Gly Tyr Gly Ala
      130              135              140

His Tyr Gln Gly Val Tyr Pro Val Lys Cys Asn Gln Asp Arg Phe Val
      145              150              155              160

Val Glu Asp Ile Val Lys Phe Gly Ser Pro Phe Arg Phe Gly Leu Glu
              165              170              175

Ala Gly Ser Lys Pro Glu Leu Leu Leu Ala Met Ser Cys Leu Cys Lys
      180              185              190

Gly Ser Ala Glu Gly Leu Leu Val Cys Asn Gly Phe Lys Asp Ala Glu
      195              200              205

Tyr Ile Ser Leu Ala Leu Val Ala Arg Lys Leu Met Leu Asn Thr Val
      210              215              220

Ile Val Leu Glu Gln Glu Glu Glu Leu Asp Leu Val Ile Asp Ile Ser

```

225	230	235	240
His Lys Met Ala Val Arg Pro Val Ile Gly Leu Arg Ala Lys Leu Arg			
245	250	255	
Thr Lys His Ser Gly His Phe Gly Ser Thr Ser Gly Glu Lys Gly Lys			
260	265	270	
Phe Gly Leu Thr Thr Thr Gln Ile Val Arg Val Val Lys Lys Leu Glu			
275	280	285	
Glu Ser Gly Met Leu Asp Cys Leu Gln Leu Leu His Phe His Ile Gly			
290	295	300	
Ser Gln Ile Pro Ser Thr Gly Leu Leu Ala Asp Gly Val Gly Glu Ala			
305	310	315	320
Ala Gln Ile Tyr Cys Glu Leu Val Arg Leu Gly Ala Gly Met Lys Phe			
325	330	335	
Ile Asp Ile Gly Gly Gly Leu Gly Ile Asp Tyr Asp Gly Thr Lys Ser			
340	345	350	
Cys Asp Ser Asp Val Ser Val Gly Tyr Gly Ile Gln Glu Tyr Ala Ser			
355	360	365	
Ala Val Val Gln Ala Val Gln Tyr Val Cys Asp Arg Lys Gly Val Lys			
370	375	380	
His Pro Val Ile Cys Ser Glu Ser Gly Arg Ala Ile Val Ser His His			
385	390	395	400
Ser Ile Leu Ile Phe Glu Ala Val Ser Ala Ser Ser His Ser Cys Ser			
405	410	415	
Ser Ser His Leu Ser Ser Gly Gly Leu Gln Ser Met Ala Glu Thr Leu			
420	425	430	
Asn Glu Asp Ala Leu Ala Asp Tyr Arg Asn Leu Ser Ala Ala Ala Val			
435	440	445	
Arg Gly Glu Tyr Glu Thr Cys Val Leu Tyr Ser Asp Gln Leu Lys Gln			
450	455	460	
Arg Cys Val Asp Gln Phe Lys Glu Gly Ser Leu Gly Ile Glu His Leu			
465	470	475	480
Ala Ala Val Asp Ser Ile Cys Asp Phe Val Ser Lys Ala Met Gly Ala			

	485						490						495					
Ala Asp Pro Val Arg Thr Tyr His Val Asn Leu Ser Ile Phe Thr Ser 500 505 510	Ile Pro Asp Phe Trp Ala Phe Gly Gln Leu Phe Pro Ile Val Pro Ile 515 520 525	His Arg Leu Asp Glu Lys Pro Ala Val Arg Gly Ile Leu Ser Asp Leu 530 535 540	Thr Cys Asp Ser Asp Gly Lys Val Asp Lys Phe Ile Gly Gly Glu Ser 545 550 555 560	Ser Leu Pro Leu His Glu Leu Gly Ser Asn Gly Asp Gly Gly Gly Tyr 565 570 575	Tyr Leu Gly Met Phe Leu Gly Gly Ala Tyr Glu Glu Ala Leu Gly Gly 580 585 590	Leu His Asn Leu Phe Gly Gly Pro Ser Val Val Arg Val Val Gln Ser 595 600 605	Asp Ser Ala His Ser Phe Ala Met Thr Arg Ser Val Pro Gly Pro Ser 610 615 620	Cys Ala Asp Val Leu Arg Ala Met Gln His Glu Pro Glu Leu Met Phe 625 630 635 640	Glu Thr Leu Lys His Arg Ala Glu Glu Phe Leu Glu Gln Glu Asp Asp 645 650 655	Lys Gly Leu Ala Val Glu Ser Leu Ala Ser Ser Val Ala Gln Ser Phe 660 665 670	His Asn Met Pro Tyr Leu Val Ala Pro Ser Ser Cys Arg Phe Thr Ala 675 680 685	Ala Thr Asp Asn Asn Gly Gly Tyr Asn Tyr Tyr Tyr Ser Asp Glu Asn 690 695 700	Ala Ala Asp Ser Ala Thr Gly Glu Asp Glu Ile Trp Ser Tyr Cys Thr 705 710 715 720	Ala				

<210> 23

<211> 2695

<212> DNA

<213> Plant

<400> 23

```

ttcacgttct cttctcaatt cccataaaaag aaacccttcc gttaggtttc cgtcctatatt 60
tctcttcttc tacgcttcct cttctgatat caatatctgt atggtgtttt tcttggttca 120
attttagatt tgttttgccct ttaataacctg taaccttata attctctgtt taaacccaaa 180
acttagcttc ttctgaagtc aggggtggga tatttgatc gtgtaagagt gtgtagaag 240
gtgattatct tttgattcag ttcctttttt gcttcttttg agggggtagc cggggcctcg 300
gcctcgcgcg gttttaatag ccccatcta ttacaacat tgggcaaaaa catcattaaa 360
tctgtacaaa gcaaaccctt aatttagttt aattttctgt attctttgat tctttaacag 420
aagaagaaga gatgccggcc ctagggtgtt gcgtagacgc tactgtttcc cctcctctcg 480
gctatgcctt ctctcgggat agctctcttc ccgcgcgga gttctttacc tccggcgtag 540
ctcctacaaa ctccgccgcc ggttccattg ggtctcggga tctgtcctct gctttgtacg 600
gggtcgatgg gtggggagct ccttatttct ccgttaactc taacggagat atctccgtcc 660
gaccacatgg tacggacaca ctccccacc aggaattga cttctcaag gtcgtgaaaa 720
aggcctccga ccgaaaaat tcaggggggc tcgggcttca gctgcctctt gttgttcgct 780
tccctgatgt gctaaaaaac cggttggaat ctctgcaatc ggcttttgat ctgctgttc 840
attcccaggg ctatggggcc cactaccaag gtgtttatcc cgtgaaatgc aatcaagaca 900
ggttcgtggt ggaagatatt gtcaaattcg ggtcgtcatt ccggttcggg ttggaagctg 960
ggtctaaacc cgagctcctg ttagccatga gctgtctctg caggggcagt gctgagggcc 1020
ttctcgtttg caatggttc aaggacgctg agtacatttc gcttgctttg gttgcaagaa 1080
agctcatggt aaacactgta attgttcttg aacaagagga ggagcttgac cttgtgattg 1140
atataagccg taagatggct gttcgccccg taattggact tcgggctaag ctcaggacca 1200
agcattcagg ccattttgga tccacttctg gagaaaaagg taagtgtggg cttacaacga 1260
cccaaattgt tcgtgtagtg aagaagctgg aagaatccgg aatgctggat tgccttcagt 1320
tgctgcattt tcacattgga tctcagatcc cttcaacggc gttgcttgct gatggtgtg 1380
gtgaggctgc tcagatttat tgtgaattaa tccgtcttg tgcgggatg aagttcattg 1440
atactggagg tgggctcgga attgattatg atggtactaa atcatgtgat tcagatgtct 1500
ctgttggtta tggcattcaa gaatagcct ccacagttgt ccaggcgggt caatatgttt 1560
gcgaccgtaa gggcgtgaag caccagtgga tttgcagcga aagtggcagg gcaattgttt 1620
ctcatcactc aattctgatt ttogaagccg tgtctgcttc tagtactca tgttcttctt 1680
cacatctgtc ttctggtggc ctccaatcca tggcggagac gctcaatgaa gatgcccttg 1740
ctgattaccg caatttatct gctgctgcag ttcgtggaga gtacgagacg tgtgtacttt 1800
actctgatca gttgaaacag agatgtgtgg atcagtttaa agaagggtcc ttgggtattg 1860
aacatcttgc tgctgttgat agcatctgtg attttgtatc aaaggctatg ggggctgctg 1920
atcctatccg cacttaccat gtgaatctgt caattttcac ttcaattcct gatttttggg 1980
cctttggtca attgtttccg attgttccaa tacaccgttt agatgaaaag cctgcagtaa 2040
ggggaatatt atcggacttg acttgtgaca gtgatgggaa ggttgataag ttcattggtg 2100
gcgaatcaag cttgcagctg catgaattgg gaagtaatgg cgatgggtgg gggtattatc 2160
tggggatggt tttgggtggg gcttatgagg aggcgctcgg aggactccac aacctgtttg 2220
gtggaccaag cgtggtgcgc gtggtgcaga gcgatagcgc tcacagcttc gccatgtctc 2280
gtcccgctcc tggcccgctc tgcgcggagc tgctccgagc gatgcagcac gagcccgagc 2340
tcatgttcga gactctcaag caccgtgcgg aggaattctt ggaacaagaa gaagacaaag 2400
ggctggccat tgcattcttg gccagcagct tagctcagtc cttccataac atgccttacc 2460
ttgtggcgcc tgcattcttg tgcttcactg cagttactgc taacaacggt ggctataact 2520

```

actattacag tgatgagaat gcagcagatt ctgctacagg ggaggatgag atttggtcct 2580
 attgcactgc ttgaagtgtt gtcgtagcat ctccagtttt agtttgtcgt cgaagttgtc 2640
 tgtttttgaa taataccctt agttggtgat gtttttctaa aaaaaaaaaa aaaaa 2695

<210> 24
 <211> 720
 <212> PRT
 <213> Plant

<400> 24

Met Pro Ala Leu Gly Cys Cys Val Asp Ala Thr Val Ser Pro Pro Leu
 1 5 10 15

Gly Tyr Ala Phe Ser Arg Asp Ser Ser Leu Pro Ala Pro Glu Phe Phe
 20 25 30

Thr Ser Gly Val Pro Pro Thr Asn Ser Ala Ala Gly Ser Ile Gly Ser
 35 40 45

Pro Asp Leu Ser Ser Ala Leu Tyr Gly Val Asp Gly Trp Gly Ala Pro
 50 55 60

Tyr Phe Ser Val Asn Ser Asn Gly Asp Ile Ser Val Arg Pro His Gly
 65 70 75 80

Thr Asp Thr Leu Pro His Gln Glu Ile Asp Leu Leu Lys Val Val Lys
 85 90 95

Lys Ala Ser Asp Pro Lys Asn Ser Gly Gly Leu Gly Leu Gln Leu Pro
 100 105 110

Leu Val Val Arg Phe Pro Asp Val Leu Lys Asn Arg Leu Glu Ser Leu
 115 120 125

Gln Ser Ala Phe Asp Leu Ala Val His Ser Gln Gly Tyr Gly Ala His
 130 135 140

Tyr Gln Gly Val Tyr Pro Val Lys Cys Asn Gln Asp Arg Phe Val Val
 145 150 155 160

Glu Asp Ile Val Lys Phe Gly Ser Ser Phe Arg Phe Gly Leu Glu Ala
 165 170 175

Gly Ser Lys Pro Glu Leu Leu Leu Ala Met Ser Cys Leu Cys Arg Gly
 180 185 190

Ser Ala Glu Gly Leu Leu Val Cys Asn Gly Phe Lys Asp Ala Glu Tyr
 195 200 205

Ile Ser Leu Ala Leu Val Ala Arg Lys Leu Met Leu Asn Thr Val Ile
 210 215 220
 Val Leu Glu Gln Glu Glu Glu Leu Asp Leu Val Ile Asp Ile Ser Arg
 225 230 235 240
 Lys Met Ala Val Arg Pro Val Ile Gly Leu Arg Ala Lys Leu Arg Thr
 245 250 255
 Lys His Ser Gly His Phe Gly Ser Thr Ser Gly Glu Lys Gly Lys Phe
 260 265 270
 Gly Leu Thr Thr Thr Gln Ile Val Arg Val Val Lys Lys Leu Glu Glu
 275 280 285
 Ser Gly Met Leu Asp Cys Leu Gln Leu Leu His Phe His Ile Gly Ser
 290 295 300
 Gln Ile Pro Ser Thr Ala Leu Leu Ala Asp Gly Val Gly Glu Ala Ala
 305 310 315 320
 Gln Ile Tyr Cys Glu Leu Ile Arg Leu Gly Ala Gly Met Lys Phe Ile
 325 330 335
 Asp Thr Gly Gly Gly Leu Gly Ile Asp Tyr Asp Gly Thr Lys Ser Cys
 340 345 350
 Asp Ser Asp Val Ser Val Gly Tyr Gly Ile Gln Glu Tyr Ala Ser Thr
 355 360 365
 Val Val Gln Ala Val Gln Tyr Val Cys Asp Arg Lys Gly Val Lys His
 370 375 380
 Pro Val Ile Cys Ser Glu Ser Gly Arg Ala Ile Val Ser His His Ser
 385 390 395 400
 Ile Leu Ile Phe Glu Ala Val Ser Ala Ser Ser His Ser Cys Ser Ser
 405 410 415
 Ser His Leu Ser Ser Gly Gly Leu Gln Ser Met Ala Glu Thr Leu Asn
 420 425 430
 Glu Asp Ala Leu Ala Asp Tyr Arg Asn Leu Ser Ala Ala Ala Val Arg
 435 440 445
 Gly Glu Tyr Glu Thr Cys Val Leu Tyr Ser Asp Gln Leu Lys Gln Arg
 450 455 460

Cys Val Asp Gln Phe Lys Glu Gly Ser Leu Gly Ile Glu His Leu Ala
 465 470 475 480
 Ala Val Asp Ser Ile Cys Asp Phe Val Ser Lys Ala Met Gly Ala Ala
 485 490 495
 Asp Pro Ile Arg Thr Tyr His Val Asn Leu Ser Ile Phe Thr Ser Ile
 500 505 510
 Pro Asp Phe Trp Ala Phe Gly Gln Leu Phe Pro Ile Val Pro Ile His
 515 520 525
 Arg Leu Asp Glu Lys Pro Ala Val Arg Gly Ile Leu Ser Asp Leu Thr
 530 535 540
 Cys Asp Ser Asp Gly Lys Val Asp Lys Phe Ile Gly Gly Glu Ser Ser
 545 550 555 560
 Leu Gln Leu His Glu Leu Gly Ser Asn Gly Asp Gly Gly Gly Tyr Tyr
 565 570 575
 Leu Gly Met Phe Leu Gly Gly Ala Tyr Glu Glu Ala Leu Gly Gly Leu
 580 585 590
 His Asn Leu Phe Gly Gly Pro Ser Val Val Arg Val Val Gln Ser Asp
 595 600 605
 Ser Ala His Ser Phe Ala Met Ser Arg Ser Val Pro Gly Pro Ser Cys
 610 615 620
 Ala Asp Val Leu Arg Ala Met Gln His Glu Pro Glu Leu Met Phe Glu
 625 630 635 640
 Thr Leu Lys His Arg Ala Glu Glu Phe Leu Glu Gln Glu Glu Asp Lys
 645 650 655
 Gly Leu Ala Ile Ala Ser Leu Ala Ser Ser Leu Ala Gln Ser Phe His
 660 665 670
 Asn Met Pro Tyr Leu Val Ala Pro Ala Ser Cys Cys Phe Thr Ala Val
 675 680 685
 Thr Ala Asn Asn Gly Gly Tyr Asn Tyr Tyr Tyr Ser Asp Glu Asn Ala
 690 695 700
 Ala Asp Ser Ala Thr Gly Glu Asp Glu Ile Trp Ser Tyr Cys Thr Ala
 705 710 715 720

<210> 25
 <211> 914
 <212> DNA
 <213> Plant

<400> 25

```

aagctcgann ttaancctca ntaaaggga caaaagctgg taccgnggcc cccctcgag 60
gtcgacggta tcgataagct tgattaagct tagtangcac attagcagcg cttgggatga 120
ttttaggcgc ggcctattcc ctttgctat ataatcgtgt ggttctggga attaaaacc 180
gatttcctcc ataaattctc cgatctaaat ggcanagaat ttccatattt ataccttttc 240
ttgttgagtg tgtttgatg ggtgtttacc ccaaagtgtt cctgggactg catgcataca 300
tccgtaagta acttaagtgc aacatggaaa atttcattga gaggaatcag caaaaaaaaa 360
aagcttaatc gaattcctgc agcccggggg atccactagt tctagagcgg ccgccaccgc 420
ggtggagctc caattcgccc tatagtgagt cgtattacaa ttcactgggc cgtcgtttta 480
caacgtcgtg actgggaaan gcctggcgtt accaacttaa tcgccttgca gcacatcccc 540
ctttcgccag ctgggcgtaa tagcgaanag gccgcacgat cgccttccca acagttgcgc 600
acctgatggn gaattggacn gccctgtanc ncgcganga ncgcggcngg tgtggtggtg 660
ccccancgtg acgcnaactt gcaacgccta acgccgcnc tgccttctcc ttcttctngc 720
aagttcncgg ctttcgtaa gtccaagcgg gggnccttag gttcgattat gttnggaccc 780
ccccnaaact gttanggtnt gtgatttggc accccaaaac gtttcccttg nctggtcntt 840
ttaaangcct ntcaacngaa accaccatcg gnacttggtta aggnthcctt gcntgnaaaa 900
nngtaaatta nntn 914

```

<210> 26
 <211> 829
 <212> DNA
 <213> Plant

<400> 26

```

agcaagctcg atatcgccct cactaaaggg aacaaaaact ggtaccgggc cccctcga 60
ggtcgacggg atcgataagc ttgattaanc ttttttttt tgaactacac aagggaattt 120
cttctcctna gtaacacatg agaataatta gtgcaataaa ttacaagagg aacattgcag 180
ttggatttaa gaatctgcgc tggggaattt agcctcaata tttgctacaa ccgtacagat 240
ttcactgcat tcatgaacga tagtatccgt gacacatcct tttggatgcc gtcctgtcca 300
catatgccac tactcacatc cactccattg ggtttaagtt gcagaaagag cttcacaaac 360
attctccggg ttaattcctc ctgccaagag ccaccatgt ttgcttctaa tgcgcggcag 420
cttaaaactga acccagttga atcctttgcc actgccacct tttgacttat ccactagaac 480
ccaatcaacc agagaagact cctcatcaga aatatagttc aaaaggctcc tcttcatttg 540
catgaagtac gtatattaca cgtttttccc tgaccaaagc ttaatcgaat tctgcagccc 600
gggggatcng gnattctaga gcggcgccac gcggtggagc tccaatcgcc taaatgancn 660
ataaaatcac tggccgtcgt ttanacgncn ggacgggaaa cctgggtacc aacttaatcg 720
cctgnagcna tccccttcnc agcggngtan acgaaaggcc gncgattgcc tccanattgc 780
cacnggatgg aanggacncc gtncgganga acngggggnn ggggtaccn 829

```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12450

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A01H 5/00; C07H 21/04; C12N 5/14, 15/29, 15/52, 15/82 US CL : 435/320.1, 414, 419; 536/23.2, 23.6, 24.5; 800/278, 317.3 According to International Patent Classification (IPC) or to both national classification and IPC																				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/320.1, 414, 419; 536/23.2, 23.6, 24.5; 800/278, 317.3 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Extra Sheet.																				
C. DOCUMENTS CONSIDERED TO BE RELEVANT																				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
X --- Y	HASHIMOTO et al. Intraspecific Variability of the Tandem Repeats in Nicotiana Putrescine N-methyltransferase. Plant Molecular Biology. 1998, Vol. 37, pages 25-37, especially Figure 3.	12 ---- 15,16																		
X --- Y	HIBI et al. Gene Expression in Tobacco Low-Nicotine Mutants. The Plant Cell. May 1994, Vol. 6, pages 723-735, especially Figure 3.	12 ---- 15,16																		
X --- Y	IZHAKI et al. A Petunia cDNA Encoding S-Adenosylmethionine Synthetase. Plant Physiology. 1995, Vol. 108, pages 841-842, see entire article.	12 ---- 15,16																		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																				
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>*T</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A document defining the general state of the art which is not considered to be of particular relevance</td> <td>*X</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*E earlier document published on or after the international filing date</td> <td>*Y</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>*G</td> <td>document member of the same patent family</td> </tr> <tr> <td>*O document referring to an oral disclosure, use, exhibition or other means</td> <td></td> <td></td> </tr> <tr> <td>*P document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A document defining the general state of the art which is not considered to be of particular relevance	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*E earlier document published on or after the international filing date	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G	document member of the same patent family	*O document referring to an oral disclosure, use, exhibition or other means			*P document published prior to the international filing date but later than the priority date claimed		
* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention																		
*A document defining the general state of the art which is not considered to be of particular relevance	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone																		
*E earlier document published on or after the international filing date	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art																		
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G	document member of the same patent family																		
*O document referring to an oral disclosure, use, exhibition or other means																				
*P document published prior to the international filing date but later than the priority date claimed																				
Date of the actual completion of the international search 17 AUGUST 2000		Date of mailing of the international search report 04 OCT 2000																		
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer AMY NELSON Telephone No. (703) 308-0196																		

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12450

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	LAMATTINA et al. RNA Editing of the Transcript Coding for Subunit 4 of NADH Dehydrogenase in Wheat Mitochondria: Uneven Distribution of the Editing Sites Among the Four Exons. Nucleic Acids Research 1991, Vol. 19, No. 12, pages 3275-3282, especially Figure 4.	12 ---- 15,16
X --- Y	LI et al. Arabidopsis Phosphoribosylanthranilate Isomerase: Molecular Genetic Analysis of Triplicate Tryptophan Pathway Genes. The Plant Cell. April 1995, Vol. 7, pages 447-461, especially Figure 3, page 459.	12,15 ---- 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12450

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-15,18-20
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest



The additional search fees were accompanied by the applicant's protest.



No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/12450

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

STN, AGRICOLA, CAPLUS, BIOSIS, EMBASE, USPAT

search terms: putrescine methyltransferase, adenosylmethionine synthetase, ornithine decarboxylase, arginine decarboxylase, NADH dehydrogenase, phosphoribosylanthranilate isomerase, DNA, cDNA, gene, nucleic

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-16, drawn to coding DNA, vector, host cell, transgenic plant.

Group II, claim(s) 17, drawn to protein.

Group III, claim(s) 18-20, drawn to transformation method and transgenic plant with promoter DNA.

The inventions listed as Groups I, II, and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The coding DNA of Group I, e.g. Claim 12, is disclosed in the prior art publication of Hashimoto *et al.* (Plant Mol. Biol. 37: 25-37, 1998; see Fig. 3b). Therefore, there is no special technical feature which links the coding DNA of Group I with the protein of Group II.

Furthermore, there is no special technical feature under PCT Rule 13.2 which links the coding DNA of Group I and the transformation method and transgenic plant with the promoter DNA of Group III. Therefore, the inventions of Groups I, II, and III do not relate to a single inventive concept under PCT Rule 13.1.